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BIOLOGY OF PUGET SOUND MARINE MAMMALS AND MARINE BIRDS: POPULATION HEALTH AND EVIDENCE OF POLLUTION EFFECTS

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EXECUTIVE SUMMARY

The objective of this research was to determine whether detrimental effects possibly caused by toxic chemicals could be observed in Puget Sound marine mammals and marine birds. The study design was based on examination of a wide variety of indices of population and individual health and comparison of these indices from areas of suspected high contaminant levels (target areas) to those from areas of suspected low contaminant levels (reference areas) and to those reported by other researchers.

Recent research conducted in Puget Sound has revealed high levels of contaminants in fish, marine mammals, and marine birds. High contaminant levels in Puget Sound have been correlated to abnormalities and disorders in fish. Researchers have also reported correlations between contaminants and disorders in marine mammals and marine birds in other parts of the world.

Primary species considered here are harbor seal, Glaucous-winged Gull, Great Blue Heron, and Pigeon Guillemot; these species were chosen because they reside, feed, and breed in some of the most contaminated portions of Puget Sound. Three other mammal species (killer whale, harbor porpoise, and river otter) were chosen as secondary study species either because they seasonally occur in contaminated areas of Puget Sound or they were found through previous research to be experiencing problems that might be pollutant-related.

Target areas were chosen that were as close as possible to the heavily contaminated Elliott and Commencement Bays. Reference areas used for different species included sites north of Puget Sound, in the Hood Canal, and in Grays Harbor and Willapa Bay on the Washington outer coast.

Several biological parameters were examined to evaluate the evidence for pollutant-related problems. These parameters included population distribution, population trends, measures of reproductive success, mortality, causes of death, incidence of gross and histological pathology, and incidence of physical deformities or disorders seen in the population. To detect a broad range of possible effects, both general population health as well as incidence of individual disorders previously linked to contaminants were examined and measured.

Study methods varied by species. Censuses were made from the ground, boats, and from small aircraft and provided population figures for several species. Reproductive rates were determined from censuses and for birds, by observing marked nests. Mortality and the incidence of reproductive disorders were determined from breeding area searches and censuses made during the breeding season. Gross pathology was determined from necropsies of animals found dead and for some species from collected individuals. Samples for histopathology, microbiology, blood parameters, and eggshell thickness were collected from appropriate species and examined by specialists for comparison between locations.

Tissues for contaminant analysis were collected and archived, but not analyzed.

We found a wide variety of disorders in some marine mammal and marine bird species. Some of these disorders followed patterns consistent with known patterns of contaminant concentrations in Puget Sound. Evidence for possible pollutant-related disorders is summarized in Table 1.

Overall, populations of the study species are doing very well, with the exception of the harbor porpoise and the killer whale; these two populations have declined in the Puget Sound area. For harbor porpoise the decline occurred prior to the 1970s and for killer whales the evidence of a decline is recent.

Harbor seal numbers are generally increasing in all our study areas, however, an unusually high incidence of premature births and neonatal mortality was found at some sites. These disorders are similar to those that have been linked to effects of contaminants in pinnipeds from other parts of the world. The high incidence of these disorders seen in Puget Sound seals, however, was found not at sites with suspected high contaminant levels but, rather unexpectedly, at study sites with suspected low contaminant levels. The most likely explanation for these problems is disease agents in these populations compounded by the possibility that these areas are nearing carrying capacity for seal numbers. A significantly higher incidence of pelage disorders and umbilical lesions seen in contaminated areas shows a pattern that suggests a relationship with contaminants.

Puget Sound marine bird populations do not appear to be declining or experiencing major reproductive problems. Two of three primary study species (Glaucous-winged Gull and Great Blue Heron), however, did show significant eggshell thinning compared to pre-1947 measurements. In addition, Pigeon Guillemots had lower overall eggshell thickness than those reported for pre-1947, but the difference was not statistically significant. These findings suggest a possible persistent detrimental effect of the pesticide DDT (and its primary metabolite, DDE) as has been reported for a wide variety of bird species in other areas. We also found evidence of highly variable liver weights in gulls that may have been associated with contaminants. No conclusive statements regarding the association between contaminants and eggshell thinning or liver weights can be made until chemical analyses of archived tissues are conducted.

Contaminant analysis of archived samples would greatly augment this study in several ways. This analysis is important in order to: 1) verify our assumptions about contaminant exposure of different populations; 2) provide a better comparison with findings in other areas; 3) allow correlation tests between disorders found in specific animals and their contaminant burdens; and 4) describe trends in recent contaminant exposure of marine mammals and marine birds.

Table 1. Summary of evidence for biological disorders possibly related to pollutants in Puget Sound marine mammals and marine birds. Except where noted data are based on biological observations in Puget Sound in 1984.

Parameter	Marine mammals				Marine birds		
	Harbor seal	Killer whale	Harbor porpoise	River otter	Glaucous-winged Gull	Great Blue Heron	Pigeon Guillemot
Current Puget Sound observations							
Population distr.	+	–	+	–	–	–	–
Population trends	–	+	+	–	–	–	–
Repro. rates	–	?			–	–	?
Mortality	–	+			–	–	–
Liver pathology	–				+[a]		–
Parasites	?				–		–
Other external pathology	+				–		
Eggshell thinning					+	+	–
Historical Puget Sound							
Population trends	+	–	+		–	–	–
Repro. rates	+	–			?		
Other parts of the world							
Population trends	+		+	+	+[b]	+	
Repro. rates	+			+	+[b]	+	
Mortality	+				+[b]	+	
Egg thinning					+[b]	+	+[b]

+ Pattern consistent with possible contaminant effect. For Puget Sound, determination is based on geographical occurrence. For other areas, determination based on reports of other researchers.
- No biological evidence for contaminant effect found.
? Evidence ambiguous.
(blank) Data lacking or inadequate.
[a] Enlarged livers.
[b] Includes studies on other congeneric species.

Biology of Puget Sound Marine Mammals and Marine Birds: Population Health and Evidence for Pollution Effects

John Calambokidis, Steven M. Speich, John Peard, Gretchen H. Steiger, James C. Cubbage, D. Michael Fry, and Linda J. Lowenstein

1. INTRODUCTION

This study reports whether detrimental effects possibly caused by toxic chemicals are detected in populations of marine mammals and marine birds in Puget Sound. Our approach was to examine population trends, reproductive success, mortality, and incidence of pathology in several species of marine mammals and marine birds in Puget Sound. The study design was based on comparisons between populations from contaminated portions of Puget Sound (target areas) and those from less contaminated areas (reference areas) and, where available, those described in the literature. We focused on species that live year-round and breed in or near contaminated portions of Puget Sound and would therefore be expected to be at greatest risk from contaminant exposure and also to be unlikely to have been exposed to contaminants elsewhere.

In recent years many toxic chemicals have been identified in samples of sediments and marine biota in Puget Sound (Malins et al., 1982; Long, 1982; Konasewich et al., 1982; Calambokidis et al., 1984; Riley et al., 1983; Mowrer et al., 1977). Bottom-fish from contaminated areas of Puget Sound have been found to have a variety of biological abnormalities including liver tumors and lesions that were statistically correlated with high levels of certain contaminants (Malins et al., 1982). Sediments and water samples from contaminated areas of Puget Sound have been shown to cause respiratory anomalies and chromosomal damage through sublethal toxicity tests with marine organisms (Chapman et al., 1982).

Marine mammals and marine birds, occupying high trophic levels, are exposed to higher concentrations of some chemical contaminants than most other marine life. Both marine mammals and marine birds feed in the marine ecosystem which receives many toxic chemicals discharged from industries and cities. Marine mammals are also long-lived and have large blubber layers that provide a depository for fat-soluble pollutants.

High concentrations of some contaminants, including PCBs and DDT, have been found in Puget Sound marine mammals and marine birds. Harbor seals from southern Puget Sound collected in the early to mid-1970s contained extremely high concentrations of PCBs in blubber (Calambokidis et al., 1984; Arndt, 1973). DDT levels in one of two killer whale (Orcinus orca) samples were also extremely high. Riley et al. (1983) reported finding PCBs in a small sample of marine birds from Puget Sound, including "very high" concentrations in a Pigeon Guillemot egg collected near Seattle.

Pollutants, particularly PCBs and DDT, have been linked to reproductive problems and population declines in a number of species of marine mammals and birds. In marine mammals, PCBs or DDT have been linked to premature births in California sea lions (Zalophus californianus) in the California Channel Islands (DeLong et al., 1973; Gilmartin et al., 1976), high mortality and reproductive failure in harbor seals (Phoca vitulina) in the European Wadden Sea (Reijnders, 1981, 1982; Reijnders et al., 1981), and pathological changes in the uterus of three species of phocid seals in the Baltic Sea (Helle et al., 1976a, 1976b; Olsson, 1978). Documented effects of pollutants on wild bird populations are far more extensive than for marine mammals with the largest number of investigations centered on the pesticide DDT (Cooke, 1973; Ohlendorf et al., 1978).

Because of the diversity in approaches required to study different species, this report is organized by species. Shorter sections are included for some species that were not primary study species but for which we gathered some information relevant to pollutant impacts.

2. GENERAL APPROACH

Four species of marine mammals and marine birds were chosen for primary study: harbor seal (Phoca vitulina), Glaucous-winged Gull (Larus glaucescens), Great Blue Heron (Ardea herodias), and Pigeon Guillemot (Cepphus columba). These species were chosen because they all live and breed in some of the more contaminated portions of Puget Sound. Secondary study species, including killer whales (Orcinus orca), harbor porpoise (Phocoena phocoena), and river otter (Lutra canadensis) were examined because these species either contain high residue levels of contaminants in Puget Sound or there was evidence that these species had experienced problems possibly related to contaminants.

The study design was based on comparisons of biological data between areas of suspected high contaminant concentrations (target areas) and areas of suspected low contaminant concentrations (reference areas). The basic assumption of the study design was that if contaminant-related disorders were occurring in marine mammals or marine birds in Puget Sound then these disorders would be expected to occur at a higher frequency in the target areas. Contaminant levels appear to be highest in marine mammals from the southern portion of Puget Sound and the Hood Canal compared to areas north of Puget Sound and on the outer coast of Washington (Calambokidis et al., 1984; Arndt, 1973). Long (1982) summarized available knowledge on contaminants in Puget Sound and concluded that contaminants were largely concentrated in the urban bays including Elliot and Commencement Bays. Target study sites were chosen in or near these urban bays in central and southern Puget Sound. Reference sites were chosen in the Strait of Juan de Fuca and on the Washington outer coast. This report covers research conducted between 5 October 1983 and 31 May 1985.

A variety of biological parameters as well as incidence of pathology were compared between geographical areas to evaluate the evidence for possible contaminant-related effects. Common to all or most of the study species were comparative evaluations of population status and trends, reproductive success, gross pathology, and histology. For harbor seals, additional parameters were compared between target and reference areas including: the length distribution of animals; the incidence of premature births and other neonatal mortality; and the incidence of pelage disorders and skin lesions. For the three marine bird study species, additional parameters compared included: degree of eggshell thinning compared to historical records; hatching and fledging success; and breeding chronology. Hematological data on Glaucous-winged Gulls were also compared.

The biological parameters we examined were chosen to represent a broad measure of population health. Special emphasis was placed on the search for disorders that were similar to those which have been linked to contaminants in marine mammals and marine birds in other parts of the world. Population changes were examined because they are the most general and broadest possible measure of the health of the population. Reproductive success is one of the more sensitive measures of population health and has been shown to be affected by many contaminants (Stendell, 1976; Radcliff, 1970; Peakall et al., 1972; Helle et al., 1976a, 1976b; Peakall, 1970). Contaminant-induced pathology has been documented in numerous species for thousands of contaminants; Puget Sound fish have pathological lesions and growths that appear to be linked to contaminants

(Malins et al., 1982). Contaminants have been linked to premature births, neonatal mortality, and skin lesions in pinnipeds from other parts of the world (DeLong et al., 1973; Gilmartin et al., 1976; Reijnders, 1980, 1981, 1982) making these important to examine in Puget Sound marine mammals. Eggshell thinning and hatching and fledging success were examined in marine birds because of the documented widespread effect of contaminants on these parameters in birds (Ratcliffe, 1970; Peakall, 1975; Risebrough et al., 1971).

A limited number of parameters was compared for the secondary marine mammal study species, killer whale, harbor porpoise, and river otter. Population trends, reproductive rates, and mortality were compared between killer whale groups suspected to be exposed to high contaminant levels and those suspected to be exposed to relatively low contaminant levels. The distribution and abundance of harbor porpoise was compared between Puget Sound and areas north of Puget Sound. For river otter, trapping records for urban/industrial counties were compared with rural counties.

3. HARBOR SEAL

The harbor seal (Phoca vitulina) was chosen as the primary marine mammal study species for several reasons: 1) it is the most common and abundant marine mammal inhabiting Washington waters (Scheffer and Slipp, 1944); 2) it is the only marine mammal that lives year-round and breeds in large numbers in Puget Sound (Everitt et al., 1980); 3) harbor seals occur throughout the coastal and protected waters of the state including southern Puget Sound (Calambokidis et al., 1979); 4) high contaminant levels have been reported in Puget Sound harbor seals (Calambokidis et al., 1984; Arndt, 1973); and 5) previous research on harbor seals from southern Puget Sound has indicated high rates of birth defects (Newby, 1973) and high neonatal mortality (Gearin and DeLong, 1984; Calambokidis et al., 1978) possibly related to contaminants.

Harbor seals have been extensively studied in Washington State. Scheffer (1928a, 1928b) conducted the earliest research on harbor seals in the state and Scheffer and Slipp (1944) provided the first comprehensive review of the biology of the harbor seal in Washington. The largest concentration of harbor seals in southern Puget Sound occurs at Gertrude Island and there have been numerous research projects at this site since 1965 (Arnold, 1968; Newby, 1971, 1973a, 1973b; Johnson and Jeffries, 1977; Skidmore and Babson, 1981; Gearin and DeLong, 1984). An examination of population size, reproduction, and mortality of Puget Sound harbor seals was done by Calambokidis et al. (1978). Thus, a large body of background information and historical population data exists for the harbor seal in Washington State.

The approach for the harbor seal research was to compare a number of indicators of possible contaminant effects in seals from areas of inferred high and low contamination (target versus reference areas). The following indicators were compared between target and reference areas: 1) population trends since 1977; 2) birth rates of harbor seals; 3) incidence of premature births and neonatal mortality; 4) differences in causes of death of neonates, subadults, and adults; 5) incidence of pathological disorders, discerned through histology, virology, and bacteriology; 6) size-frequency distribution of harbor seals; and 7) incidence of pelage and skin disorders.

3.1 Harbor Seal Study Methods

3.1.1 Study sites and land census effort

Harbor seals were censused primarily from land observation sites at study areas in southern Puget Sound, Hood Canal, and areas north of Puget Sound. Figure 1 shows the location of study sites and Table 2 lists the census effort at each site. The timing of seal counts, observation locations, and census techniques were comparable to the methods used at these same sites in 1977 and reported in Calambokidis et al. (1978).

During a visit to a study site, censuses were conducted every half hour and recorded on data sheets along with information on weather conditions. Censuses were generally conducted with 20-60X spotting scopes and binoculars. Visits to study sites were timed to bracket the time of

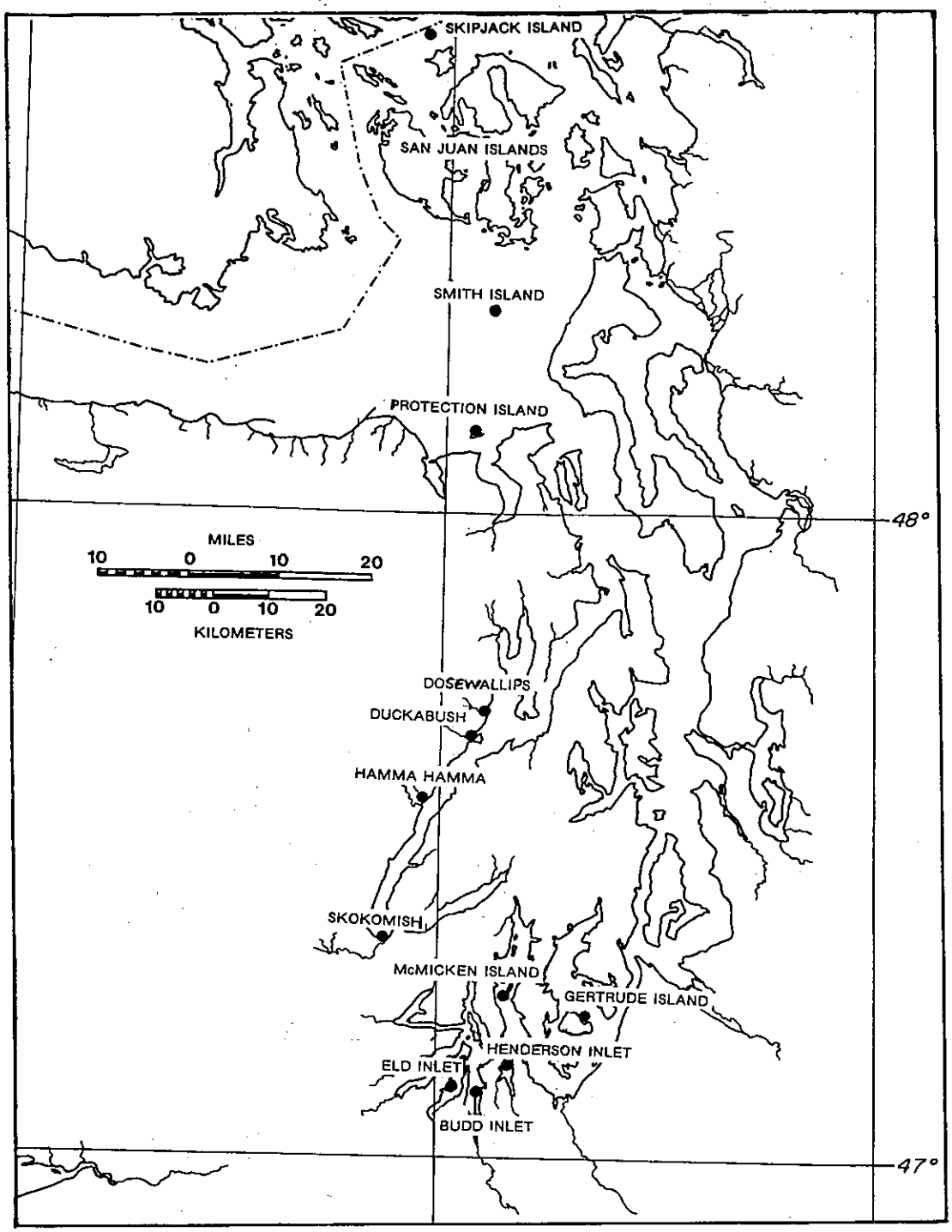

Figure 1. Harbor seal study sites in western Washington, 1984.

Table 2. Time spent by Cascadia personnel censusing harbor seals at principal study sites in 1984. See Appendix A for more detailed information on activities at study sites.

Sites	Land counts		Aerial counts
	Days counts made	Hours counting	
Southern Puget Sound			
Gertrude Island	73	359	9
Henderson Inlet	55	130	8
Budd Inlet	57	81	5
Eld Inlet	46	46	8
McMicken Is.	5	11	6
Total SPS	236	627	
Hood Canal			
Skokomish Delta	53	160	7
Duckabush Delta	42	70	4
Dosewallips Delta	41	59	4
Hamma Hamma Delta	24	11	5
Quilcene Bay	3	1	4
Total HC	163	301	
North of Puget Sound			
Smith Island	48	420	7
Protection Is.	30	44	6
Skipjack Is.	6	19	4
Total NPS	84	483	
All locations	483	1411	

expected peak number of seals (Calambokidis et al., 1978; Johnson and Jeffries, 1977).

Within each region (southern Puget Sound, Hood Canal, and north of Puget Sound) we identified one site as a primary study site where both census effort and ground searches (see Section 3.1.3) were concentrated. Other sites within each region were monitored less frequently than the primary study sites. A description of the sites we monitored in these regions and our census effort follows.

Southern Puget Sound. All sites within this region were considered target sites because of high levels of contaminants found in seals from this area (Calambokidis et al., 1984). This region was defined as the portion of Puget Sound south of the Tacoma Narrows. Gertrude Island, a small islet connected to McNeil Island at low tide, was our primary study site in this region. Located in Still Harbor, Gertrude Island is a part of the McNeil Island State Penitentiary. It is the largest seal haul-out area in southern Puget Sound. Unlike seals at other haul-out areas, seals using Gertrude Island are protected from disturbance caused by boaters and by the general public because the McNeil Island area is closed to the public. Censuses were generally conducted either from an observation tower on the spit where the seals hauled or from the SE shore of Still Harbor on McNeil Island, opposite Gertrude Island. Censuses were carried out near the time of low tide; when peak numbers of seals occur at this site.

Additional land censuses of secondary study sites in southern Puget Sound were conducted at Henderson, Budd, and Eld Inlets and at McMicken Island. Henderson Inlet is the second largest seal haul-out area in southern Puget Sound. Seals at this site haul out on logs independent of tide at a commercial log storage facility. Activities of the log boom operation made counts difficult during weekdays when work at the site was in progress. We conducted censuses primarily on weekends, as we did in 1977, to avoid interference from the log boom operation. Counts were made from a railroad trestle adjacent to the seal haul-out area. Seals in Budd Inlet also haul out on logs. We censused this site from a point about 50 m above the site. Censuses were made in the morning hours before workers began moving logs. Harbor seals in Eld Inlet haul out at night and in early morning hours on recreational floats scattered throughout the inlet. We counted seals at this site during the early morning hours from several locations that provided a good view of different portions of the inlet. A network of Eld Inlet residents who lived within sight of floats used by seals provided us with additional counts. Seals at McMicken Island were observed either from a point above the haul-out area or from a boat anchored offshore. Seals at this site haul out primarily at night and in the early morning.

Hood Canal. All study sites in the Hood Canal were considered secondary target areas because seals in this area appear to be exposed to intermediate concentrations of contaminants (Calambokidis et al., 1984). Our primary study site in the Hood Canal was the Skokomish River Delta, with secondary study sites at the Duckabush and Dosewallips River Deltas. Seals at all these sites haul out on marshes at high tide and our censuses were conducted primarily around high tide. All of these sites were difficult to observe from the land because there was no single location that was close to the haul-out site, provided a good view of all areas used

by seals, and had adequate elevation to make accurate counts of seals. At Skokomish Delta counts were made from adjacent marsh areas as well from a ridge, 200 m high, located 1 km from the haul-out area. Censuses at Dosewallips were made both from a distant bluff and from a tree on the marsh near the haul-out site. Censuses at Duckabush were made from several locations including the top of a highway bridge. The other major seal haul-out areas on the Hood Canal, in Quilcene Bay and at the Hamma Hamma River Delta, were counted opportunistically.

North of Puget Sound. Study sites north of Puget Sound served as reference areas because of the lower level of contaminants likely to occur in this area compared to target areas. Our primary site in the area north of Puget Sound was Smith Island, in the Strait of Juan de Fuca. Smith Island is connected to Minor Island at low tide by a gravel spit. These two islands will be referred to together as Smith Island, which, as a whole, are a wildlife refuge that is closed to the public. Observations at Smith Island were made primarily from the west end of the island because its higher elevation provided a view of the major seal haul-out areas. Seals at this site do not have a clearly defined haul-out cycle, so hauled animals were counted throughout the day. Seals that hauled on the eastern portion of Minor Island were not always visible from our census locations. Complete counts could be conducted only from a boat or during aerial surveys.

Secondary sites monitored in this area include Skipjack Island in the San Juan Islands and Protection Island in the Strait of Juan de Fuca. Censuses at Protection Island were made from bluffs overlooking both Violet Point, on the east end, and Kanem Point, on the west end of the island. Skipjack Island was monitored primarily to gather data on age class, sex, and pelage of seals (see Section 3.1.8) because this observation site provides a close view of the seals.

3.1.2 Aerial surveys

Aerial surveys were flown during 21 days between 27 April and 15 November 1984. The primary purpose of these surveys was to cover all the major haul-out areas in a region during the same haul-out period to avoid any possibility of duplicate counts of seals. We treated southern Puget Sound, Hood Canal, and north of Puget Sound as three distinct regions because of their relative geographical separation. Southern Puget Sound aerial surveys included all known haul-out areas south of Tacoma. The Hood Canal included all known haul-out areas south of Pt. Gamble. North of Puget Sound included all haul-out areas in Washington waters in the Strait of Juan de Fuca east of Dungeness, including the San Juan Islands, and Bellingham, Samish, and Padilla Bays.

Four aerial surveys were conducted of all the major haul-out sites both in the Hood Canal and north of Puget Sound. Five aerial surveys covering all haul-out areas in southern Puget Sound were flown. In addition to these surveys, other flights covered only portions of the haul-out sites in a particular region. Table 2 shows the number of surveys flown over different study sites.

All surveys were flown in a Cessna 172 or 182, a single-engine high-wing aircraft, out of Olympia, Washington. Generally three Cascadia

personnel and the pilot flew on aerial surveys. The photographer flew in the front right seat; an observer responsible for aerial counts of harbor seals flew in the back right seat; and the recorder flew in the back left seat.

A Nikon camera with a 200-mm lens and color print film was used to photograph seals for counts when groups of greater than 50 seals were hauled (see Section 3.1.9 for more details on photographic techniques). Photographs were made without obstruction through the opened right window of the plane, and counts were made subsequently from prints. Aerial surveys were generally flown at approximately 200 m over the haul-out areas. Counts from the air were made while the plane was making right hand slow circles, thus keeping the seals in constant view to the observers.

3.1.3 Recovery of dead marine mammals

To assure recovery of as many dead marine mammals as possible, we regularly searched harbor seal haul-out areas, participated in the Northwest Marine Mammal Stranding Network, and made contacts with people who were likely to find dead animals (Appendix Table A-1).

Searches. Beach searches were conducted regularly at all of the study sites to look for dead pups and birth sites (Table 3, for more detail see Appendix Table A-2). One or more persons walked the haul-out and surrounding areas. Additional areas were checked by skiff cruises near shore, using binoculars to scan for carcasses. Searches were timed to create as little disturbance as possible; areas were searched when no seals or a small number of seals were hauled. The location of all dead pups and birth sites found were recorded. All dead pups were collected and necropsied, and all evidence of births was removed to prevent duplication.

Information about the location and timing of births was used in determining pupping phenology and minimum birth rates. Harbor seals give birth on land, often leaving afterbirth remains at the birth site. These remains include the placenta or portions of the placenta and the lanugo pelage shed by the pup in utero and expelled with the placenta. A birth site was identified by clumps of lanugo pelage which were often accompanied by blood. Fresh placentas were frequently removed from the birth site by birds which prey on portions of them. Placentas found were not counted as a birth site unless no other evidence of lanugo or blood were observed in the area at that time.

Searches began several months prior to the pupping season and continued though the end of the season until after mother-pup pairs were no longer observed at the site or after several weeks of finding no dead pups or birth sites (see Table 3 for dates for each site).

Stranding network. We were notified of and responded to marine mammal strandings in our study region as a part of the Northwest Stranding Network. Calls from the general public were reported to a stranding response center, usually via the Washington State Patrol. Participants in the network with whom we have worked cooperatively include the National Marine Fisheries Service in Seattle, the Marine Animal Resource Center (MARC) in Seattle, the Washington State Department of Game in Olympia, and the Moclips Cetological Society in Friday Harbor.

Table 3. Searches conducted for dead harbor seal pups and birth sites at study sites in 1984. Dates listed are the ranges for each site.

Site	Dates	# searches	Total hrs.
Southern Puget Sound			
Gertrude Island	17 May - 21 Nov	70	76
Henderson Inlet	5 Jul - 27 Aug	13	24
Eld Inlet	8 Jul - 11 Aug	6	13
McMicken Is.	6 Mar - 8 Aug	10	5
Total SPS		99	118
Hood Canal			
Skokomish Delta	15 Jan - 7 Nov	27	65
Duckabush Delta	11 Feb - 27 Sept	22	20
Dosewallips Delta	19 Apr - 27 Sept	18	29
Total HC		67	114
North of Puget Sound			
Smith Is.	4 May - 29 Aug	45	63
Protection Is.*	20 May - 29 Aug	28	13
Skipjack Is.	6 May - 13 Aug	5	3
Total NPS		78	79
All locations		244	311

* Does not include 14 searches conducted for us by Walter Reed.

Other contacts: Additional dead animals were reported by people who live or work in areas where dead seals are likely to be found. We talked to workers at log booms where seals haul out, workers at marinas, and people living near Puget Sound (Appendix Table A-2).

3.1.4 Reproductive timing and rate

The timing of harbor seal pupping at different sites was designated as beginning on the date the first nursing pup was seen or the date we first found evidence that a birth had occurred (placenta, fetal sack, or lanugo hair). See Section 3.1.3 for our search effort and procedure at seal haul-out areas. The termination of the pupping season was designated as the last date a birth site was found. The termination of the pupping season also could be determined indirectly from counts of pups and by subtracting four weeks from the date the last nursing pup was seen, as four weeks is the average nursing period.

Two procedures were used to determine the crude birth rate of seals at each site (total birth rate and sampled birth rate). We calculated a total birth rate for harbor seals at different sites from the high count of pups during the pupping season, plus the number of pups that had died before that date and the number of births (from birth sites) that occurred after that date.

The sampled birth rate was determined at sites where we frequently observed seals at a distance of <100 m. At these sites we could determine the sex and general age class of seals that were resting with their ventrum towards the observer. The sex of a seal was determined from the presence of a penile opening or nipples on the ventral surface. Reproductive condition of adult females (pregnant, with pup, or neither) was determined for females sampled directly before and during the pupping season. The sampled birth rate was determined by taking the number of females that were pregnant or with a pup as a percent of the total non-pup animals sampled.

3.1.5 Necropsy procedure

All necropsies followed a standard procedure (described in detail in Appendix B). All animals were examined for gross abnormalities and probable cause of death. One hundred forty-two harbor seals were examined: 16 adults, 13 subadults, 110 pups, and 3 fetuses (Appendix Tables A-3 and A-4 describe the number of harbor seals sampled and the number of samples collected).

Tissue samples were collected for chlorinated hydrocarbon analysis, heavy metal analysis, Leptospirosis analysis, virology, general bacteriology, histopathology, and protein analysis (Appendix Tables A-3 to A-5). The extent to which we could thoroughly sample an animal depended upon its condition.

Sampling for future analyses for chlorinated hydrocarbons. Blubber, muscle, liver, brain, and stomach contents were placed in glass jars. If the animals were in poor condition, brain tissue was often not sampled. For animals that were extremely decomposed, only blubber was sampled. Stomach contents were collected from a number of animals from southern Puget Sound.

Sampling for future analyses for heavy metals. Liver, kidney, and spleen were collected into plastic whirl pacs. If these tissues were not distinguishable due to decomposition, samples were not taken.

Virology. The nose, throat, and anus of all fresh pups were sampled into medium using a dacron swab. This type of sampling was implemented after we began finding a large number of premature pups on Smith Island.

Leptospirosis sampling. Sampling for Leptospirosis also began after we found a large number of premature pups on Smith Island. Liver, kidney, and urine were sampled into a Leptospirosis medium from all fresh pups. Fresh placentas were sampled when found.

Bacteriology. The brain, liver, and respiratory fluid (when present) were sampled using a culturette swab for all fresh animals.

Histopathology. Numerous tissues were collected into 10% buffered formalin. Only fresh or nearly fresh animals were sampled.

3.1.6 Histology

Samples were collected for histology from 74 harbor seals as described in Section 3.1.5. A subset of tissues of 51 pups and 4 adults and subadults were submitted to Dr. David Gribble of Evergreen Professional Services, Kirkland, Washington, for examination. Samples selected represented those that appeared to be in the best condition and included the majority of the samples available from all regions and for all age classes. Samples were examined following standard histological methods.

3.1.7 Microbiology

Specimens collected to determine the presence of viruses and Leptospirosis (described in Section 3.1.5) were sent to the College of Veterinary Medicine at Oregon State University, and were examined under direction of Dr. Alvin W. Smith and Dr. Douglas E. Skilling.

Virology. Samples for virus isolation were processed immediately upon receipt of specimens at Oregon State University, or were frozen at -85°C to await preparation of appropriate cell cultures. Swabs (nasal, throat, or anal) in 2-dram vials containing tissue culture media were vortexed and then clarified by centrifugation at 2,000 x g for 10 minutes. A 0.2-ml aliquot of the resulting supernatant was placed in each of 3 roller tubes containing monolayers of Vero Monkey Kidney, Porcine Kidney, or Crandall Feline Kidney Cells. After 1 hour absorption of the "dry" monolayer, tubes were refed with 1.5 ml of MEM containing 2% FBS and antibiotics. Tubes were incubated at 37°C on a roller drum and were observed daily by light microscopy for CPE. Cultures demonstrating 3+ to 4+ cytopathic effect were freeze-thawed, vortexed, clarified and repassaged. Cultures not showing CPE were frozen after 10 days; then thawed, vortexed, clarified by low-speed centrifugation, and repassaged. Each culture was passaged at least 3 times on each cell line.

Twenty-four randomly selected virology specimens (original swab samples) were processed for negative stain electron microscopy as described in Skilling et al. (1985).

Leptospirosis. Tissue samples (kidney, liver, placenta) and fluids for possible leptospiral isolation were processed immediately upon receipt of specimens, using methods described in Smith et al. (1974a). Tissue was ground in a sterile Ten Broeck grinder, diluted with PBS, and inoculated into tubes containing EMJH semisolid medium. Cultures were incubated at 30°C and were periodically examined by darkfield microscopy. At the time of preparation, a drop of each ground tissue was also observed by darkfield microscopy for the presence of leptospires.

Bacteriology. Bacterial culturettes were examined for identification of bacterial isolates at the Veterinary Diagnostic Laboratory of Oregon State University.

3.1.8 Ventral and observational sampling

We recorded the incidence of different pelage types, scarring patterns, and behavior at sites where seals could be observed close enough to determine sex. This method for determining age and sex of animals and the birth rate was discussed in Section 3.1.4. Each animal was scored for all categories allowing cross-indexing of different categories (i.e. pelage type by sex).

Pelage type was scored in one of five categories: 1) light--a light grey or silver base with dark spots, 2) dark--predominately black base even on ventrum with white spots or rings, 3) indistinct--a gray coat with no distinct spotting, 4) mixed--a coat between light and dark where the ventrum has a checkerboard appearance, and 5) red--seals with a distinct reddish coloration.

Scars or wounds on seals were tallied for two locations: the head and neck region and the area around the umbilicus. These areas were chosen because of the high incidence of lesions and scars in these locations. The umbilical region was scored as: 1) normal--umbilicus barely discernible through pelage, 2) enlarged or swollen umbilicus--umbilicus swollen or enlarged and clearly discernible, 3) scarred--umbilicus shows clear evidence of scarring or healed lesion present, and 4) ulcerated--open sore or lesion present.

3.1.9 Photogrammetry

Seals were photographed from the air and measured with photogrammetric techniques to discern age-class population structure of major haul-out sites. Vertical photos of haul-out areas were taken with a hand-held 35-mm Nikon FE camera equipped with a motor drive, 200-mm fixed-focal-length lens, and an electronic level that audibly signaled the photographer when the camera back was within 5 degrees of parallel to the ground. Altitude of aircraft was recorded to the nearest 10 ft. Aircraft were slowed to 60-80 kt over the target and held at constant altitude of approximately 200 m while the photographer leaned out of the right window and photographed downward. Clearance between struts and wheel fairings allowed vertical photography. Film was either 160 ISO Vericolor III or 400 ISO Fujicolor color-negative film. Photo scale was derived either from targets of known size in the photo frame (at Skokomish Delta we placed a target array on the marsh; in Eld Inlet, the sizes of floats were measured), or

with barometric altitude calibrated with photo measurements of known-size objects adjacent to the hauling ground.

Images on the negatives were measured on a stereo dissecting microscope (25x) equipped with an ocular reticle calibrated with a stage micrometer that allowed measurements to the nearest 0.04mm. Seals in corresponding prints of negatives were numbered to minimize redundant measurements of seals occurring in more than one photograph. The lens was calibrated to 203.0 mm using methods described in Wolf (1983). Symmetrical radial distortion was less that 0.06 mm across the format. Known-size objects near the major hauling grounds (dock at Gertrude, jetboat at Protection Island marina, foghorn building at Minor Island, parking lot stripes near Dosewallips and Duckabush Deltas) were measured on ground and photographed during aerial surveys. The mean difference between the expected scale based on altitude and focal length and the measured scale based on the ratio of image and object sizes provided a correction for the altimeter-based scale in those photos where no known-sized objects were in the frame.

The equation used to measure seals was:

$$\text{Seal size} = \text{image size} \left(\text{altitude}/\text{CFL}\right) + \text{Correction}$$

where CFL is calibrated focal length and Correction is based on the mean difference of altimeter-based scale (altitude/CFL) and measured scale (object size/image size) of photos of known-size objects taken adjacent to the haul-out area on the same survey as the seals were measured. The average scale of the photographs was around 1:1,000.

Seal images and measurements were graded according to how bent the animal was (flex) and image resolution. Flex was graded from 1 to 4 with 1 being straight and 4 unacceptable flex. Resolution and contrast were graded from A to F, with D being estimable and F being unacceptable. Measurements on seals were made from the tip of the snout to the end of the rear flippers.

To minimize variation in seal measurements, only those measurements of seals with an overall degree of flex of quality 1 or 2 were analyzed. Only resolution grades A, B, and C were used. Table 4 reviews the number of photos, surveys and sites that were measured.

To test the precision of the altimeter, 3 sets of 4 photos were taken at varying altitudes on different days. The resulting regressions of measured scale on altitude-based scale were highly significant ($p<.001$) and none had a slope significantly different than 1 ($p>.05$). To test the variance and get an estimate of accuracy of the altitude-based scale measurement, all those instances where more than one photo was taken of a ground target on one survey was grouped into a set. One of each set of these photos was chosen at random to provide a scale correction for the rest of the photos in the same set. With these scale corrections, photogrammetric measurements were made and compared with true measurements. Mean percent difference of measurements from true size was +0.15% (n=11, s.d.=1.33%, range -2.8% to 1.7%) or presented differently, the mean difference was 0.019 m on an average of a 5.31-m long target (s.d.=0.0743,

Table 4. Sites, frequency, and number of photographs of harbor seal haul-out areas studied with photogrammetry.

Region	Site	# of photo surveys	# of photos analyzed	# of seals measured*
S. Puget Sound	Gertrude Island	2	11	303
	Eld Inlet**	2	7	17
	Henderson Inlet	1	2	12
Hood Canal	Skokomish Delta**	2	4	64
	Duckabush Delta	1	2	38
N. of Puget Sound	Smith Island	4	14	299
	Protection Island	1	3	73

* Count of analyzed seals with flex grade of 1 or 2 and resolution grade of A,B, or C (see Section 3.1.9 for explanation).

** Scale determined through measurement of known-sized object in photograph. In all other photos, scale was determined with barometric altimeter readings and calibrated focal length of the lens.

coefficient of variation = 1.4%). Thus, the 95% confidence of the mean of scale accuracy (not including error in seal measurement) is +/- 0.89%.

To test for additional error in seal measurement, individual seals photographed in two photos were paired and the measurements compared. Only seals with similar grading (flex grade 2 and resolution better than grade 3) were compared. The mean difference between measurements of seals was 0.036 m (s.d.=0.038, n=25) between the first and second photos and the average seal size in the first was 1.37 m. If the two sets of measurements are averaged, the average difference is +/- 1.3% of the average length.

3.2 Results and Discussion

3.2.1 Census and distribution

Results of all harbor seal censuses for each of our study regions are given in Appendix Tables A-6 to A-8. These tables summarize survey effort, high count of seals and pups, and the average daily high count for censuses that included the time of expected peak numbers. Other than our primary study sites, counts at all other sites are based mainly on aerial surveys where we surveyed all possible haul-out areas within our study regions. Results of the aerial surveys summarized by region are given in Appendix Tables A-9 and A-10.

We did not locate any major seal haul-out sites in central Puget Sound, near or between Elliott and Commencement Bays. These areas are among the most heavily contaminated in Puget Sound and would have been the ideal target study areas for evaluating the possible effects of contaminants on harbor seals. Our previous research, reports from the literature, discussion with other researchers, and surveys in these areas, provided evidence for only limited numbers of seals hauling out in this region. This gap in the distribution of harbor seals indicates an historical or present factor is preventing seals from using this area to the degree found in areas immediately north and south. A number of factors including contaminants, habitat destruction, human disturbance of seals at haul-out areas, and historical hunting pressure could be responsible for the low numbers of seals in these areas.

3.2.2 Changes over time in numbers of seals

One of the primary objectives of censuses was to estimate the change in number of seals that have occurred in recent years at different sites. Changes would indicate to what degree harbor seals in different areas might be responding to the negative effects of pollution or to other factors, including positive circumstances. The best, most consistent sample results are for individual haul-out areas and not for censuses of entire regions. For this reason we chose to examine changes in seal numbers for specific sites. The study sites generally comprised the majority of seals in each region. In the area north of Puget Sound a large proportion of the seal haul-out areas occur in numerous small groups in the San Juan Islands; in this region census changes were analyzed by examining all these sites together.

Previous research done by authors of this report in 1977 (Calambokidis et al., 1978) provides the most relevant data for comparison with our current results. Additional counts by Everitt et al. (1979, 1980) for the San Juan Islands and Arnold (1968), Newby (1971, 1973a), Johnson and Jeffries (1977), Skidmore and Babson (1981), and Gearin and DeLong (1984) for Gertrude Island were also used for comparison to 1984.

Three indicators were used to analyze the degree of change at different sites: the high count of seals seen in a given year, the mean of the daily high counts made during peak haul-out times, and the high count of pups. We compared high counts of seals because they tend to minimize the effect of differences in methods and effort. Pup counts were used as an additional indicator because pups can reliably and consistently be counted during the pupping season and should roughly parallel overall changes in seal numbers.

Table 5 and Figure 2 compare 1984 counts at some of the major haul-out areas in our study regions with those reported previously. Because of varying time intervals between some of the previous studies, we report census change as an annual growth rate. There was generally good agreement between the three measurements of growth at a given site. We averaged the different growth figures for a site to obtain a growth index, an overall estimate of the annual growth rate at that site.

Seal numbers increased at all monitored harbor seal haul-out areas in recent years. The mean of the peak counts was the only one of the three indices of growth that could be tested statistically for significant changes between years because a variance of this measure could be calculated. At all major study sites tested except Skokomish Delta, counts of seals were significantly higher in 1984 than in the late 1970s (t-test, $p<.05$, see Table 5). We found no significant difference in the magnitude of the site growth index between the three regions (ANOVA, $p>.05$).

The lowest growth rate (2.3%) was at Skokomish Delta, the largest haul-out area in the Hood Canal. The growth rates at the other two sites in the Hood Canal were also low compared to other areas. Not reflected in our census comparison, however, is a dramatic increase in the number of seals at Hamma Hamma Delta, a site on the Hood Canal between Skokomish and Duckabush Deltas. Although this was not a study site, counts were made at this site during aerial surveys and incidental to our counts at other sites. Up to 131 seals were seen at this site, with an average daily peak count of 76 (n=9, s.d.=37). In 1977, the highest count of seals at the Hamma Hamma Delta was nine. A log boom haul-out site at Jorsted Creek (< 2 km south of Hamma Hamma) used by up to 30 seals in 1977 was gone in 1984. Even if the seals currently hauling at Hamma Hamma are seals that previously used both Jorsted Creek and Hamma Hamma, the increase from 1977 to 1984 is substantial.

The growth rate at sites in southern Puget Sound was somewhat variable (7-31%) with the highest growth occurring at Gertrude Island and Henderson Inlet, the largest haul-out areas in southern Puget Sound. The annual growth rate for sites north of Puget Sound was more consistent and ranged between 10 and 17%.

Table 5. Changes in numbers of harbor seals at study sites. Census results from this study are compared to historical counts judged to be most similar in effort and methods to current study. Annual increase refers to yearly increase required to achieve observed growth. Mean of peak counts was calculated from the daily high counts of seals made when period of expected peak haul out was censused. Statistical significance refers to t-test between counts made in 1984 and those from previous study.

Site	Previous study Refer.	Yr	High count Previous	1984	Ann. incr.	Mean of peak counts Previous	1984	Ann. incr.	Stat. sign.	Pups born Previous	1984	Ann. incr.	Growth index
Southern Puget Sound													
Gertrude Island	1	79	220	483	17%	-	-	-		38	83	17%	17%
Henderson Inlet	2	77	40	228	28%	18	120	31%	***	8	60	33%	31%
Budd Inlet	2	77	29	59	11%	14	27	10%	***	6	8	4%	8%
Eld Inlet	2	77	30	53	8%	15	28	9%	***	11	13	2%	7%
Hood Canal													
Skokomish Delta	2	77	342	428	3%	151	165	1%	NS	-	-	-	2%
Duckabush "	2	77	163	302	9%	102	144	5%	*	21	37	9%	8%
Dosewallips "	2	77	160	339	11%	99	166	8%	***	25	29	2%	7%
North of Puget Sound													
Smith Island	2	77	245	546	12%	125	181	5%	*	41	105	14%	10%
San Juan Islands	3	78	899	1800	12%	562	1553	18%	***	-	-	-	15%
	3	79	818	1800	17%	732	1553	16%	***	-	-	-	17%
Skipjack-Bare Is.	2	77	93	216	13%	70	143	11%	***	8	16	10%	11%

References: 1--Skidmore and Babson (1981), 2--Calambokidis et al. (1978), and 3--Everitt et al. (1979, 1980)
Ann. incr.=Annual increase in seal numbers
Stat. sign.=Statistical significance (NS-not significant, *-$p<0.05$, ***-$p<0.001$)

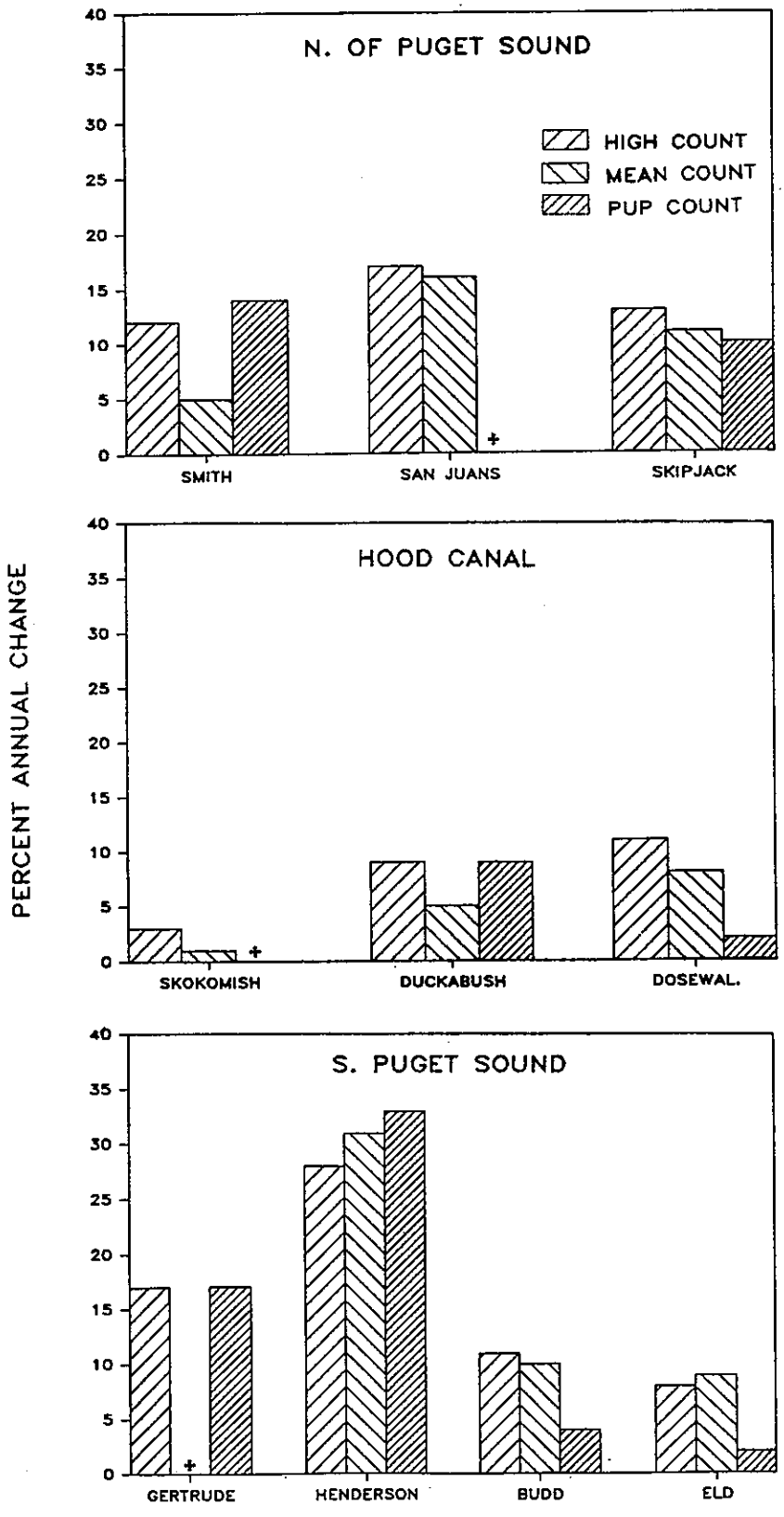

Figure 2. Increases in the number of harbor seals at haul-out sites in Washington State (summarized from Table 5). + Indicates comparable data are not available to determine changes.

The growth rate at Henderson Inlet in southern Puget Sound was anomalously high (31%). Census methods and efforts for this site in 1984 were very similar to those in 1977 (Calambokidis et al., 1978). Harbor seals at this site haul out on log rafts and are normally disturbed during the day by the activities at the site. During 1984, however, work at the site was discontinued for much of the summer, reducing disturbance to the seals. Though we monitored most of the major haul-out areas in southern Puget Sound, we did not monitor several smaller haul-out areas that are closest to Henderson Inlet. McMicken Island, a haul-out site just 12 km north of Henderson Inlet, is a State Park and heavily used by boaters in the summer. We suspect that the unusually high growth rate at Henderson Inlet was the result of seals hauling in higher numbers at Henderson during daylight and the movement of seals from adjacent areas.

Seals at Gertrude Island have been studied by a number of researchers from 1965 through 1984. Figure 3 shows the change in the high counts of seals and pups reported during this period. The number of harbor seals at Gertrude appeared to show no sign of increase through the late 1970s. Starting in 1979, an increase becomes apparent.

The degree of growth that occurred at other study sites prior to 1977 is more difficult to determine. Scheffer and Slipp (1944) report a minimum estimate of 5,000 seals for Washington State but this was a rough figure and was not broken down by site. Newby (1973a) provides an estimate of the Washington harbor seal population by site. This estimate was incomplete, however, with some major harbor seal haul-out areas known to be active at that time (such as sites in the Hood Canal) not included in his estimate. Despite these limitations, comparisons of census estimates for the San Juan Islands and Smith Island using Newby's 1972 censuses and the 1977 figures by Calambokidis et al. (1978) suggest seal numbers were increasing prior to 1977. Similarly, rough comparisons of Newby's figures for Grays Harbor and Willapa Bay with those reported by Johnson and Jeffries (1977) also suggest that substantial increases in harbor seal numbers were occurring on the outer coast of Washington State prior to 1977. Calambokidis et al. (1978) cite evidence for increases in harbor seal numbers occurring prior to 1977 in the Hood Canal.

The apparent increases in the number of harbor seals occurring in the early and mid-1970s at most locations in Washington State except Gertrude Island indicate that seals at Gertrude may have had unique problems not occurring at other sites. Our findings of rapidly increasing numbers at Gertrude Island should not, therefore, be viewed as inconsistent with the high incidence of disorders and reproductive disfunctions reported by Newby (1971) at this site. The annual increase occurring at Gertrude in recent years is among the highest we found for any site and may be the result of seal numbers held well below carrying capacity in the early and mid-1970s. Locations where seals are farthest below carrying capacity would be expected to grow the fastest.

The increase in the number of harbor seals is consistent with recent changes in marine mammal management. Until the early 1960s, the Washington Department of Fisheries paid a bounty on harbor seals because of the purported predation of seals on commercially-valuable fish. Over 10,000 harbor seals were killed under this program. All marine mammals, including harbor seals, received protection from killing and harassment from the

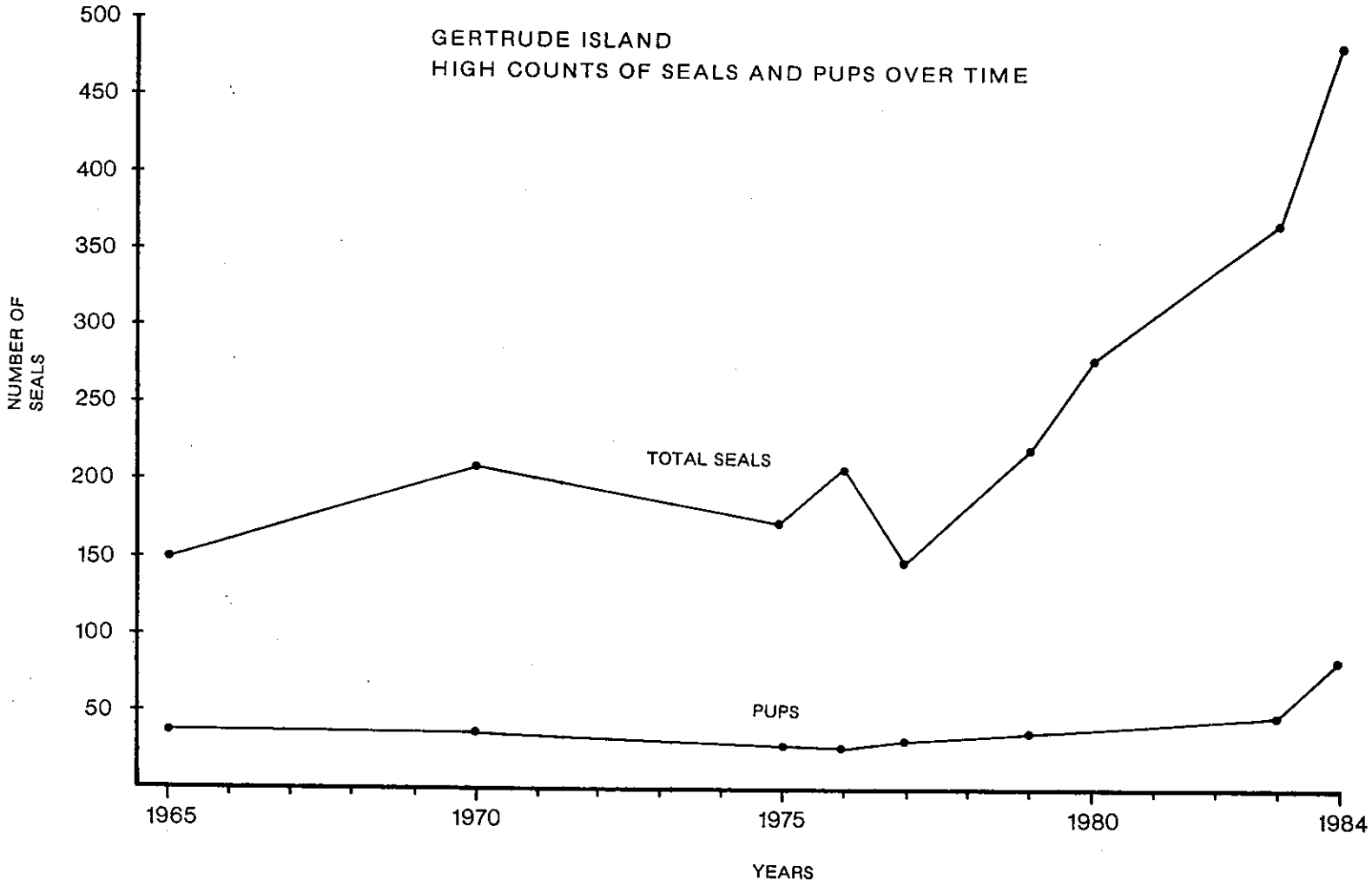

Figure 3. High counts of harbor seals and harbor seal pups at Gertrude Island in southern Puget Sound. Data from Arnold (1968), Newby (1971), Johnson and Jeffries (1983), Skidmore and Babson (1981), Gearin and DeLong (1984), and current study.

Federal Marine Mammal Protection Act of 1972. Currently, the only killing of harbor seals allowed under this legislation is by permit though illegal shooting of seals continues. An increase in the harbor seal population would be expected to occur following a reduction of seal hunting.

Though concern about the effects of contaminants on Puget Sound harbor seals prompted this research, concern about other threats to seals have also been raised. Newby (1973a) reported the abandonment of some haul-out areas by seals and suggested the effects of the bounty, contaminants, and human disturbance as responsible factors. The impact of human disturbance on seals has been reported as a potentially major threat to harbor seals in other areas (Risebrough et al., 1979; Reijnders, 1981; Drescher, 1978). The strong growth of harbor seals in southern Puget Sound, an area with high levels of some contaminants as well as a high degree of human activity, may reflect the resilience and adaptability of this species.

The high rate of increase in the number of harbor seals in Puget Sound is generally consistent with findings of rapid growth (up to 17%) in some other pinniped populations. Beach et al. (1982) report a 17% increase in harbor seal pups born in the Grays Harbor-Willapa Bay-Columbia River area between 1976 and 1981. Payne (1977) reported an annual growth rate of 17% for the Antarctic fur seal (Arctocephalus gazella) after commercial killing stopped. Our rates of annual increase of 2-17% for all sites except Henderson Inlet, which was discussed earlier, appear reasonable.

The increase in numbers of seals and their consistency with our findings on reproductive rates and mortality are discussed further in the context of a harbor seal population model in Section 3.2.9.

3.2.3 Reproduction

The reproductive success of harbor seals and the presence of reproductive disorders were examined because reproduction appears to be highly susceptible to chronic exposure to contaminants. Links between pollutants and disorders in pinnipeds from other parts of the world have all involved reproductive problems (DeLong et al., 1973; Gilmartin et al., 1976; Helle et al., 1976a, 1976b; Helle, 1980; Reijnders, 1981). The pupping season and birth rates of harbor seals in our study regions were examined for evidence of any pollutant related disorders. Reproductive problems related to premature and neonatal mortality are discussed in Section 3.2.4.

Pupping season. Table 6 shows the period of pupping for harbor seals at our study sites (see Appendix Tables A-6 to A-8 for observation dates). Pups were generally born over a 1-2 month period with nursing pups present at most sites for 2-3 months.

There were major differences in the pupping seasons of seals at different sites. Pupping occurred earliest at Smith Island, north of Puget Sound, where the first full-term pup was seen on 2 June. Pupping at Protection Island, only 22 km from Smith Island, didn't start until 11 July. Pupping in the San Juan Islands began near the first of July, though our information is incomplete. Pupping in southern Puget Sound began in early July at all our study sites except Gertrude, where pupping began on 30 July. The end of pupping was similarly staggered with Gertrude being a

Table 6. Pupping seasons of harbor seals at different study sites in 1984 measured by several techniques.

Sites	Dates nursing pups seen first	Dates nursing pups seen last	Dates birth remains found first	Dates birth remains found last	Date high count of pups w/ mothers
Southern Puget Sound					
Gertrude Island	3 Aug.	27 Oct.	30 July	21 Sept.	12 Sept.
Henderson Inlet	4 July	2 Sept.	5 July	11 Aug.	7 Aug.
Eld Inlet	16 July	26 Aug.	8 July	31 July	3 Aug.
Budd Inlet	11 July	15 Sept.	ND		5 Aug.
Hood Canal					
Skokomish	4 Aug.	7 Nov.*	7 Aug.	2 Oct.	19 Sept.
Hamma Hamma	8 Aug.	19 Sept.	ND		13 Sept.
Duckabush	27 July	27 Sept.	27 July	30 Aug.	30 Aug.
Dosewallips	27 July	27 Sept.	18 July	6 Sept.	13 Sept.
North of Puget Sound					
Smith Island	2 June	29 Aug.	2 June	12 Aug.	16 July
Protection Is.	11 July	13 Sept.	17 July	13 Aug	8 Aug.

* Last day observations made, so pups with females may have been present later.
ND Not determined

month later than the other sites in southern Puget Sound. The pupping season at sites in the Hood Canal began in late July and early August and, with the exception of Skokomish, the last pups were born at the end of August and beginning of September. Skokomish had an unusual pupping season that lacked definite peaks characteristic of other sites; pups were born at least into October when monitoring ended.

The patterns of pupping we found in 1984 closely resemble those reported by previous researchers at Gertrude Island for the 1970s (Johnson and Jeffries, 1977; Newby, 1971) and 1983 (Gearin and DeLong, 1984) and for 1977 at all our other study sites (Calambokidis et al., 1978). The variations in pupping seasons of harbor seals along the west coast of North America have been reviewed by Bigg (1969b) and Calambokidis et al. (1979). Clinal genetic patterns controlled by photoperiod have been suggested as the mechanism for a geographic pattern of pupping dates along the range of the harbor seal (Bigg, 1969b). The large and consistent differences noted in the pupping times at certain harbor seal haul-out sites in north of Puget Sound and in southern Puget Sound have yet to be explained. Genetic differences or photoperiod are not plausible explanations for this local phenomena.

The extended pupping season we found at Skokomish in 1984 is similar to the extended pupping seen in previous years at this site (Calambokidis et al., 1978). In 1977-1978 nursing pups (less than a month old) were seen in 10 months of the year at Skokomish. This extended and late pupping season has not been reported for any other harbor seal population in the world. This pupping season anomaly may be linked to low reproduction at this site as evidenced by absence of population growth (Section 3.2.2).

Contaminant concentrations have been linked to disruptions in the breeding cycle in different species. Some pollutants such as PCBs and DDT have been reported to induce hepatic microsomal enzymes that could result in increased metabolism of steroid hormones (Peakall, 1967; Conney et al., 1967). Ray and Rockwell (1982) and Peakall (1967) suggested that this type of pollutant effect might result in prolonged estrous and delay of breeding.

PCB and DDT concentrations in harbor seals from the Skokomish River Delta in 1977 were not as high as those in southern Puget Sound (Calambokidis et al., 1984). There have not been any broad spectrum analyses, however, conducted to test for high concentrations of any other contaminants at this site. We are not aware of any industrial or agricultural activities near the Skokomish Delta that could be a source of high levels of contaminants. Until tests can be conducted to examine for concentrations of other contaminants, we cannot further evaluate the possibility of contaminants playing a role in the unusual reproductive timing of pupping and lack of population growth at this site.

Birth rate. Two methods were used to determine the birth rate at different sites, total birth rate and sampled birth rate (described in Section 3.1.4). The total birth rate was calculated from census figures for all study sites and is detailed in Table 7.

Table 7. Total birth rates of harbor seals at different sites in 1984. Total pups born was calculated as the sum of the high pup count, dead pups found before the high pup count, and the number of births that occurred after the high pup count. Total birth rate was calculated as the total pups born taken as a percent of the non-pup high count.

Site	High pup count		Dead pups before count	Births after count	Total pups born	Non-pup high count	Birth rate
	date	#					
Southern Puget Sound							
Gertrude	12 Sept.	72	9	2	83	384	22%
Henderson	7 Aug.	52	8	0	60	197	30%
Eld Inlet	—	0*	2	11	13	35	37%
Budd Inlet	5 Aug.	6	2	0	8	38	21%
Combined SPS region					164	654	25%
Hood Canal							
Skokomish	22 Aug.	25**	2	46	63	315	20%
Duckabush	30 Aug.	33	4	0	37	268	14%
Dosewallips	13 Sept.	27	2	0	29	312	9.3%
Combined HC region					129	895	14%
North of Puget Sound							
Smith Is.	16 July	75	25	5	105	414	26%
Protection	8 Aug.	50	10	3	63	333	19%
Combined NPS region					168	747	22%
All locations					461	2296	20%

* Total birthsites exceeds high count of 7; birthsites used to calculate birth rate.

** High pup count of 29 not used due to the prolonged pupping time; combination of pup count and birthsites used to determine pups born.

The total birth rate by region ranged from 14% to 25% and varied significantly by region (chi-square, $p<.001$). This regional difference was primarily the result of the low overall birth rate in the Hood Canal compared to southern Puget Sound and sites north of Puget Sound. Overall southern Puget Sound had the highest pooled birth rate at 25%, followed by areas north of Puget Sound with a 22% rate, and Hood Canal with the lowest birth rate of 14%. The total birth rates, like the population increases, did not follow a pattern suggesting a link between contaminants and reproductive problems in the more contaminated southern Puget Sound seals.

Total birth rates ranged from 9 to 37% at different sites and as with the regional birth rates, varied significantly by location (chi-square, $p<.001$). When sites were tested for differences within regions only Hood Canal showed significant variation by site (chi-square, $p<.01$). The lowest total birth rates by site were at the Dosewallips (9.3%) and the Duckabush Delta (14%) in the Hood Canal. The total birth rate at Skokomish Delta was higher (20%), though the extended pupping season at this site required the use of counts of birth sites rather than counts of pups for determining the pups born.

Total birth rates for sites in southern Puget Sound were highest for seals at Henderson and Eld Inlet. Seals at both these sites use anthropogenic habitats for haul-out substrates. The high total birth rates calculated for these sites may either reflect a preference by mothers and pups for this habitat or reflect an underestimate of the total population of seals at these sites. At Eld Inlet seals haul out primarily at night and scatter in the water during the day. A similar pattern may exist at Henderson Inlet. Mothers and pups which appear to spend more time near the haul-out area may be overrepresented in the daytime counts of seals at Henderson Inlet if other seals are hauling out primarily at night. Newby (1971) suggested that seals using haul-out areas in southern Puget Sound other than Gertrude Island involved transients that breed at Gertrude. This is not the case today and these haul-out areas should be regarded as important breeding areas for harbor seals.

At Gertrude Island and Henderson Inlet, sites where we could observe seals closely, a sampled birth rate was calculated from the proportion of seals that were pregnant or with a pup (see Section 3.1.4 for details). The proportion of reproductive females (pregnant or with pup) at Gertrude Island tends to decline gradually for the period from July to September (Table 8). This would be expected since the number of adult females with pups that died or had weaned would increase during this period, thereby reducing the number of apparently-reproductively-active females sampled by this technique. Since the sampled birth rates by month for the July to September period were not significantly different from each other (chi-square, $p>.05$), the pooled value of a 23% birth rate was considered the best estimate. The sampled birth rate would be expected to be similar to the total birth rate presented earlier. The sampled birth rate of 23% at Gertrude Island is very similar to and substantiates the 22% total birth rate described earlier. The more limited data from Henderson Inlet indicate a sampled birth rate of 28%, again similar to the 30% total birth rate.

An estimate of the proportion of non-reproductive females can also be calculated from the age-sex data for Gertrude Island and Henderson Inlet

Table 8. Summary of reproductive status of adult female harbor seals at Gertrude Island and Henderson Inlet directly before and during the pupping season. Samples are for seals showing their ventral surface to the observer so as to allow sex and age class to be determined.

Period	No. adults and sub-ad.	(a) Adult fem. #	(a) Adult fem. %	(b) Preg. fem. #	(b) Preg. fem. %	(c) Fem. w/ pup #	(c) Fem. w/ pup %	b+c Reprod. fem. #	b+c Reprod. fem. %	a-(b+c) Non-reprod. fem. #	a-(b+c) Non-reprod. fem. %
Gertrude Island											
July	126	38	30	33	26	0	0	33	26	5	4
August	484	140	29	50	10	65	13	115	24	25	5
Sept. 1-15	892	275	31	39	4	165	19	204	23	71	8
Sept. 16-31	376	116	31	4	1	72	19	76	20	40	11
Total	1878	569	30	126	7	302	16	428	23	141	7
Henderson Inlet											
June - Aug.	70	29	43	4	6	15	22	19	28	10	15

Fem.=Female, Preg.=Pregnant, Reprod.=Reproductively active

(Table 8). Figure 4 shows the percent total adult females and percent females that were not pregnant or with a pup by month. The proportion of lone females increased significantly during the pupping season (chi-square, $p<.01$). This would most likely be the result of the increased number of females whose pups had died or been weaned. During July and August when very few pups had died or weaned 17% of the adult females were not pregnant or with a pup, indicating a pregnancy rate of 83% for the females judged to be mature. At Henderson Inlet, the proportion of apparently non-reproducing adult females was 34% of the total females (pregnancy rate of 66%). The low pregnancy rate at Henderson Inlet indicates the high observed birth rates at Henderson are not the result of a high pregnancy rate but of the high proportion of adult females in the sampled population.

Caution must be used in interpreting the significance of birth rates at a single site. Segregation of seals by reproductive condition has been documented in several areas. Several thousand pregnant females move from sites a minimum of 50 km away to pup on icebergs in Glacier Bay, Alaska, resulting in birth rates of 50-60% at these locations (Calambokidis et al., 1985). Similarly Beach et al. (1982) report the apparent migration of parous females from the Columbia River to Grays Harbor prior to the pupping season. This movement results in calculated birth rates of over 30% in Grays Harbor and less than 5% in the Columbia River. Though no patterns as dramatic as these are apparent from our data, some of the variation especially between sites in the same region may be the result of selective use of sites by females with pups.

The birth rate at Gertrude Island in 1983 was reported to be low (15%) compared to previous years and other locations (Gearin and DeLong, 1984). The authors raised concerns that this low birth rate might indicate the presence of reproductive problems in the Gertrude population. We found no evidence of similar problems in 1984. The birth rate in 1984 of 22% is within the range of 21-25% for birth rates from 1970 to 1979 as summarized by Gearin and DeLong (1984). The difference between the birth rates reported in 1983 and those in 1984 for Gertrude Island may be the result of: 1) unexplained differences in the reproductive rate between years, or 2) differences in sampling methods between 1983 and 1984. There is some evidence to suggest the latter may be the case. Counts during peak haul-out times in 1983 were made primarily from the southern shore of Still Harbor (Gearin and DeLong, 1984) while counts in 1984 were made from this site as well as from the observation tower and aerial survey photographs. We found pup counts especially difficult to make from Still Harbor because of the distance and lack of elevation.

The regional birth rates roughly parallel the patterns in growth rates by region reported in Section 3.2.2. Though this pattern cannot be accurately tested with only 3 regions, it is consistent with the pattern of rapid growth in southern Puget Sound and slow growth in the Hood Canal. The pattern does not match as well, however, when examined on a site by site basis. Skokomish Delta, the site showing the lowest population growth, appeared to have a normal birth rate, though the timing of pupping was extremely unusual.

Birth rates in 1984 were in the same range as those reported for many of these same sites in 1977 (Calambokidis et al., 1978). Similar to 1984, the birth rate for seals in Eld Inlet in 1977 was unusually high. The

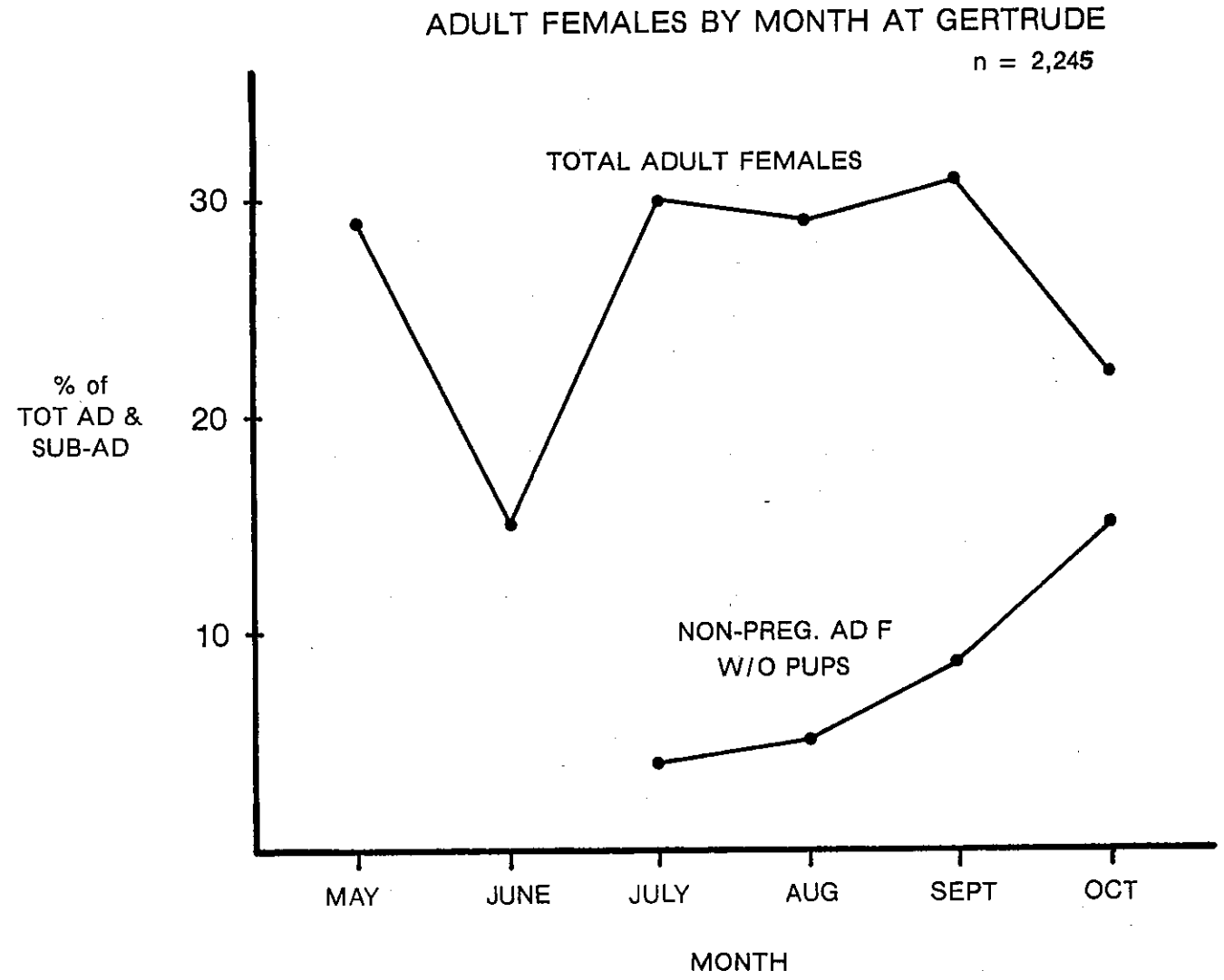

Figure 4. Adult females and non-pregnant adult females without pups as a proportion of all harbor seals other than pups sampled at Gertrude Island by month. Seals were sampled as described in Section 3.1.8.

pattern of low birth rates in the Hood Canal in 1984, however, was not seen in 1977 except possibly at the Skokomish Delta (Calambokidis et al., 1978).

The overall birth rate we found in 1984 (20%) appears to be slightly lower than that reported for harbor seals in areas outside Washington State (Bigg, 1969a; Bishop, 1967; Venables and Venables, 1955). This is the direct result of the low birth rate in the Hood Canal. We found no evidence of low birth rates in southern Puget Sound seals similar to that reported in Wadden Sea harbor seals (Reijnders, 1981), or the low reproductive rates related to uterine occlusions in Baltic Sea pinnipeds (Helle et al., 1976a, 1976b; Helle, 1980). Both sets of disorders in those areas had been correlated to PCB concentrations that are similar to the levels found in southern Puget Sound seals (Calambokidis et al., 1984).

Additionally, we found no evidence of early-term reproductive failure in the nine adult females we found dead in the course of the study (see Section 3.2.4 for a full discussion of mortality). Of the four adult females found dead in southern Puget Sound, three were either pregnant or had recently given birth. The fourth was found in poor condition in the fall (before fetal development) and so its reproductive status could not be determined.

3.2.4 Neonatal mortality and premature pupping

The incidence of neonatal mortality and premature pupping was examined because of its link to pollution in pinnipeds from other parts of the world (DeLong et al., 1973; Gilmartin et al., 1976; Reijnders, 1981). Premature births, birth defects, and a high rate of neonatal mortality were reported in harbor seals in the early 1970s on Gertrude Island in southern Puget Sound (Newby, 1971, 1973b) and were suspected to be linked to pollutants (Arndt, 1973).

Premature pupping. Table 9 lists the number of premature pups by site found during our haul-out searches. Eleven percent of the pups born were premature on both Smith Island and Protection Island, our two major study sites north of Puget Sound. The incidence of premature pupping in this region is significantly higher (chi-square, $p<.001$) than southern Puget Sound (1.8%) and Hood Canal (0.78%). Thirty-six percent of the dead pups recovered at Smith Island were premature (n=33). There was no significant difference in the rate of premature pupping among the sites within a given region (chi-square, $p>.05$).

We observed one premature pup on Smith Island with scoliosis. This was the only congenital defect observed in dead or live pups at all of our study sites.

We found a low incidence of premature pupping in southern Puget Sound. At Gertrude Island, one premature pup was recovered out of 83 pups born (1.2%) in 1984. This pup was found on 17 May 1984, 74 days before the beginning of the regular pupping season. Newby (1971, 1973b) reported premature pups as early as 14 June in 1965 and 23 May in 1970. He found 2 premature pups out of an estimated 38 pups born (5.3%) in 1970. Gearin and DeLong (1984) found no premature pups in 1983, although their observations

Table 9. Premature births, neonatal mortality, post-weaning pup mortality, and total pup mortality of harbor seals in Puget Sound as a percent of pups born in 1984. Dead pups recovered from other areas (besides our study sites) are also listed. One possible premature pup taken into captivity from Case Inlet in southern Puget Sound is not included in the table.

Site	Pups born	Premature births #	Premature births %	Neonatal deaths #	Neonatal deaths %	Post-weaning deaths #	Post-weaning deaths %	Total mortality #	Total mortality %
				MONITORED SITES					
Southern Puget Sound									
Gertrude Is.	83	1	1.2	13	16	2	2.4	16	19
Henderson In.	60	1	1.7	7	12	6*	10	14	23
Eld Inlet	13	1**	7.6	1	7.6	0	0	2	15
Budd Inlet	8	0	0	2	25	0	0	2	25
Total SPS	164	3	1.8	23	14	8	4.9	34	21
Hood Canal									
Skokomish	63	1	1.6	8	13	0	0	9	14
Duckabush	37	0	0	4	11	0	0	4	11
Dosewallips	29	0	0	2	6.9	0	0	2	6.9
Total HC	129	1	0.78	14	11	0	0	15	12
North of Puget Sound									
Smith Is.	105	12	11	20	19	1	1.0	33	31
Protection	63	7	11	4	6.3	0	0	11	17
Total NPS	168	19	11	24	14	1	0.60	44	26
All locations monitored	461	23	5.0	61	13	8	1.7	93	20
				OTHER AREAS IN REGION					
Other southern Pug. Sound		-		3	-	4	-	7	-
Central Puget Sound		-		1	-	2	-	3	-
Other Hood Canal		-		1	-	0	-	1	-
Other northern Pug. Sound		-		5	-	1	-	6	-
Outer Coast		-		1	-	0	-	1	-

* Includes five weaned pups incidentally caught in nets
** Includes premature pup reported to us from before pupping season

did not begin until 7 June. The rate of premature pupping at our two reference sites north of Puget Sound in 1984 was higher than that found at Gertrude Island in the early 1970s.

Pup mortality rate. Table 9 lists the rate of pup mortality (including premature pupping and post-weaning deaths) by site. Pup mortality by region is summarized in Figure 5. There was a significant difference between regions in the overall pup mortality rate (chi-square, $p<.05$). The rate varied from 21% at our study sites in southern Puget Sound, to 12% at our sites on the Hood Canal, to 26% at our sites north of Puget Sound. There was no significant difference in the rate of pup mortality between sites within each region (chi-square, $p>.05$). We found the highest pup mortality rate at Smith Island, where 31% of the pups born, died.

Mortality rates must be considered minimums. We found dead pups on early visits to both Smith Island and Gertrude Island indicating some mortality could have been missed early in the season. Dead pups were scavenged by Bald Eagles (Haliaeetus leucocephalus) at Smith Island and Protection Island, and by Turkey Vultures (Cathartes aura) at sites on the Hood Canal. As many as 44 Turkey Vultures were observed over the Skokomish Delta haul-out site at one time and they appeared to be responsible for the high proportion of scavenged skin and bones of pup carcasses that were found on the Hood Canal.

The behavior of pups at Smith Island, where we found a high rate of premature births and neonatal mortality, also appeared unusual. We observed an unusually large number of pups (up to 14) hauled out without mothers. These animals usually did not move during the day from the areas where we observed them. We never observed a mother returning to any of these animals. Generally, these pups were weak and would not try to enter the water when approached by an observer. They were apparently abandoned close to the time of birth; when these pups were first observed they had umbilical cords and appeared to have a normal amount of blubber. A minimum of three animals had a white-yellow milky secretion over their eyes (Corynebacterium sp. was recovered from one of the eye secretion sample). These observations of lone pups continued, and peaked on 6 July when 14 lone pups were on the beach at one time. Most of these animals were later recovered dead and emaciated.

The percentage of post-weaning deaths found at each site is also included in Table 9, which indicates the minimum mortality rate for pups after the first month of life. Recovery rates after the pupping season were better for southern Puget Sound than other areas, therefore post-weaning deaths in the Hood Canal or north of Puget Sound are probably underrepresented.

Overall neonatal mortality rates we found in harbor seals are generally similar to the high neonatal mortality reported for some other pinnipeds. Mortality rates for other pinniped pups range from several percent of the pups born to over 60% (Summers et al., 1975; Doidge et al., 1984; Mattlin, 1978; Keyes, 1965; Baker et al., 1980; Anderson et al., 1979; Le Boeuf et al., 1972; Carrick et al., 1962). Most mortality studies have been conducted on otariids and elephant and grey seals (Halichoerus grypus). These species have a more stable breeding area that is not

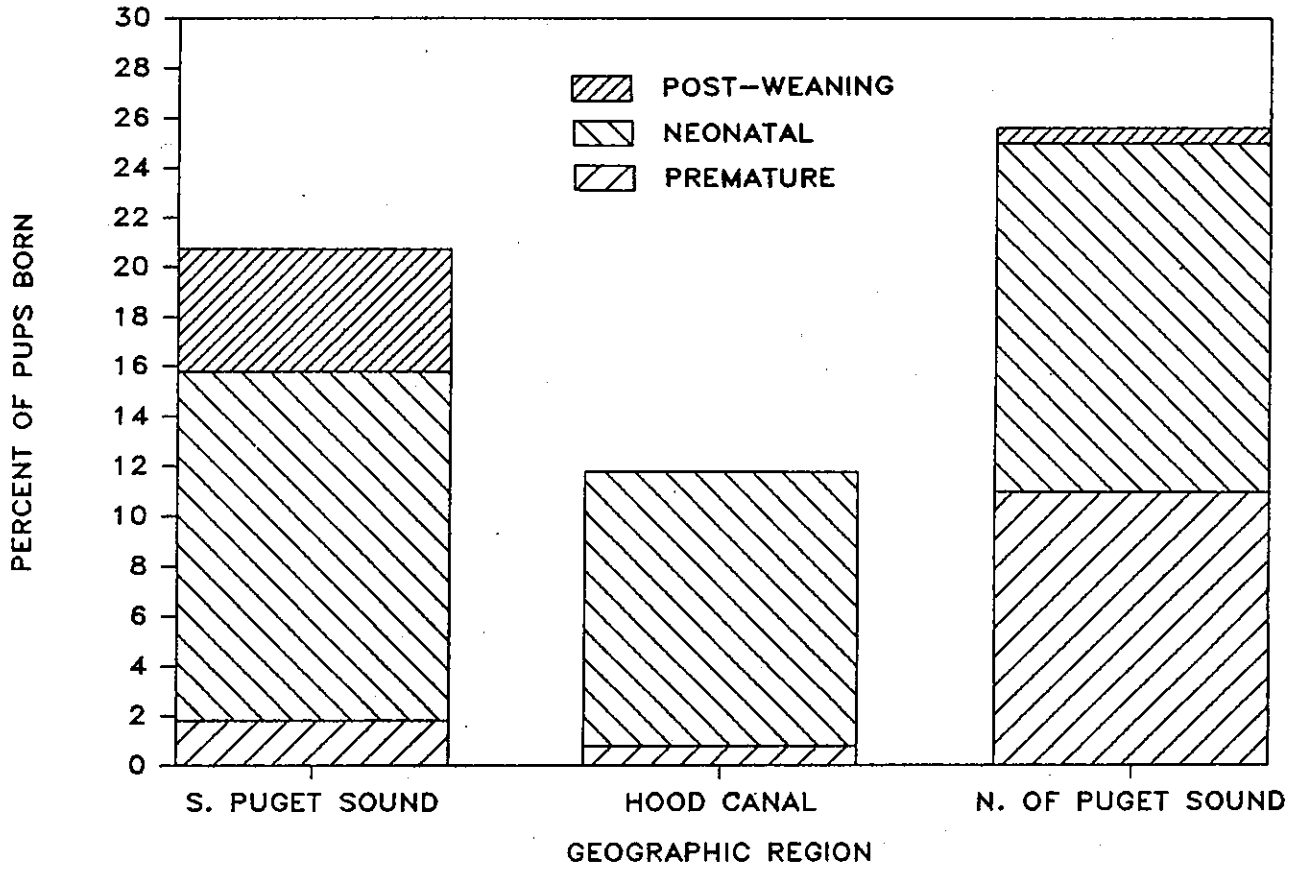

Figure 5. Mortality of harbor seal pups by region (summarized from Table 9). Premature births, neonatal, and post-weaning all refer only to the proportion of these pups that were found dead.

generally subject to tidal inundation. Documented neonatal mortality rates would therefore be expected to be more complete and higher than in harbor seals or other phocid seals that use tidal areas. In this context the 31% neonatal mortality rate we found for harbor seals on Smith Island appears high.

The number of dead pups found at Gertrude (16) was similar to the 15 found by Gearin and DeLong (1984) in 1983. As a percent of the estimated number of pups born, however, the mortality rate in 1983 is much higher because of Gearin and DeLong's lower count of pups born in 1983 compared to our 1984 counts.

The number of pups found dead at sites in Puget Sound in 1984 was much higher than that reported for many of the same sites (using similar methods) in 1977 (Calambokidis et al., 1978). This increase largely appears to be the result of the increased population size in all areas of Puget Sound (see Section 3.2.2). The overall neonatal mortality rate for 1977 was 14% compared to 20% in this study. This increase could partially be the result of the greater search effort in 1984 compared to 1977. The increase may also be the result of higher mortality rates for higher population sizes. Pup mortality has been shown to be density dependent in northern fur seals (Callorhinus ursinus)(York, 1985; Swartzman, 1984), Antarctic fur seals (Doidge et al., 1984), and grey seals (Summers et al., 1975).

There were differences between 1977 and 1984 in the pattern of neonatal mortality by site. Pup mortality rates reported for 1977 ranged from 8 to 14% of the pups born with the exception of two sites in southern Puget Sound that showed a 50% pup mortality rate. A total of only 14 pups were born at these two sites, so the 1977 mortality figures should be treated cautiously. The highest neonatal mortality in 1984 was at Smith Island, north of Puget Sound.

Cause of death. Table 10 lists the primary and contributing causes of death of harbor seal pups by region. A high incidence of premature pups and emaciated pups were recovered on Smith Island. At Gertrude Island, 7 and possibly 8 pups were killed by coyotes (Canis latrans). This was the only site where coyote predation was observed. The rate of occurrence of animals with septicemia or other infectious agents was similar in both southern Puget Sound and north of Puget Sound. Cause of death information for the Hood Canal is not comparable to the other regions due to the high number of pups that were unsuitable for examination.

Newby (1971) reported a high rate of birth defects at Gertrude Island in 1970 and suggested it could be related to high pollution levels. He observed 1 pup with a palatine shift and 3 with umbilical hernias. Johnson and Jeffries (1983) and Calambokidis et al. (1978) observed a small number of birth defects in harbor seals in southern Puget Sound in the late 1970s. Gearin and DeLong (1984) did not observe any pups at Gertrude Island with birth defects in 1983. We observed none in 1984.

The high proportion of neonatal deaths in Puget Sound seals caused by emaciation is consistent with studies of other pinnipeds. Starvation has been listed as the primary cause of neonatal death in grey seals (Bonner, 1970; Baker et al., 1980; Anderson et al., 1979), southern elephant seals

Table 10. Primary and contributing causes of death of harbor seal pups recovered between 22 Oct. 1983 and 29 Jan. 1985 by region. Three pups found dead in central Puget Sound are included with the southern Puget Sound figures. Includes one premature pup reported to us before the pupping season from Eld Inlet, and two pups from the 1983 pupping season. One pup from Grays Harbor was not included. P-Primary, C-Contributing. SPS+CPS-Central and southern Puget Sound, HC-Hood Canal, NPS-North of Puget Sound.

Cause of death	SPS+CPS		HC		NPS		All sites	
	P	C	P	C	P	C	P	C
Premature	2	1	1	0	19	0	22	1
Stillborn (not premature)	3	1	3	0	4	4	10	5
Emaciation								
pre-weaning	3	0	2	0	9	1	14	1
post-weaning	3	0	0	0	0	0	3	0
Coyote kill	7	0	0	0	0	0	7	0
Incidental catch-fisheries	5	0	0	0	0	0	5	0
Shot	1	0	0	0	1	0	2	0
Blow to head	3	0	1	0	2	0	6	0
Drowning *	2	5	0	0	0	1	2	6
Septicemia	2	3	0	0	2	3	4	6
Other suspected disease agents	0	4	0	0	1	3	1	7
Myocardial necrosis	0	0	0	0	1	0	1	0
Pneumonia	0	1	0	0	0	0	0	1
Died or euthen. in captivity	2	0	0	0	1	0	3	0
Not determined or unsuitable	11	-	9	-	10	-	30	-
Total examined	44		16		50		110	

* Does not include animals that drowned as a result of other injuries included in another category (i.e. emaciation or incidental catch).

(Mirounga leonina)(Carrick et al., 1962), northern fur seals (Callorhinus ursinus)(Keyes, 1965), New Zealand fur seals (Arctocephalus fosteri) (Mattlin, 1978), and Antarctic fur seals (Baker and Doidge, 1984). In northern elephant seals (Mirounga angustirostris) starvation occurs in conjunction with trauma apparently as a result of starvation weakened pups being more susceptible to injury from adult males and females (Le Boeuf and Briggs, 1977).

Other primary causes of death we found are similar to those reported for other species, though the proportion of animals dying from other causes varies widely between species. The rate of stillborn pups in this study generally appears higher than those reported for other pinnipeds (Carrick et al., 1962; Baker and Doidge, 1984; Mattlin, 1978). Septicemia, umbilical infections, pneumonia and other diseases have been reported as causes of death of other pinniped neonates (Anderson et al., 1979; Baker et al., 1980; Bonner, 1970; Baker and Doidge, 1984).

The seven cases of coyote predation seen at Gertrude Island also appeared to be linked to disease. Tissues of four of these pups were examined for histological disorders; three showed symptoms of septicemia or other infectious agents. Stroud and Roffe (1979) reported several cases of shark predation on seals with chronic debilitating diseases. Suspected coyote predation was described by Gearin and DeLong (1984) of at least one and possibly four harbor seal pups on Gertrude Island in 1983, but otherwise has not to our knowledge been previously reported. Predation by coyotes on pups that are suffering from serious disease indicates this predation has a less serious impact on the population than suggested by the simple examination of primary causes of death. It is also reasonable to include the mortality of these pups in our site comparisons since they would likely have died even in the absence of coyotes.

Histopathology. A summary of the significant histopathology seen in tissues of harbor seal pups examined is included in Appendix Table A-11. In addition to the lesions and pathology directly associated with the causes of death of these animals several additional patterns were noted that could not be directly linked to gross findings or the cause of death. Twelve harbor seal neonates that were sampled showed hepatic atrophy or unusual pigmentation. Ten of these animals were from Smith Island and only two were from other areas. The proportion of Smith Island neonates with this condition was significantly higher than for the neonates at our other sites (chi-square test, $p<.001$). Thymic atrophy (in animals with no other symptoms of stress) and lack of colloid in the thyroid were observed in a number of animals, but no regional variation or association with particular causes of death was seen relative to either of these conditions.

Microbiology. Microbiological results of all harbor seals sampled are summarized in Table 11. All but three of the samples examined were taken from pups. Results for individual pups are summarized in Appendix Table A-11. A wide range of bacteria were identified and there were differences by location in the bacteria seen. About half of the animals sampled from both the Hood Canal and north of Puget Sound contained Proteus sp., while only 2 of 22 samples from southern Puget Sound contained this bacteria. The significance of finding Proteus in animals that had been dead for some period is questionable. The regional difference, however, is surprising since there was not any consistent bias in how animals were sampled between

Table 11. Results of microbiological examination of harbor seals by region. Table lists number of animals from which specific bacteria or viruses were identified. All virology and Leptospira samples and all but three general bacteriology samples were from pups. See Appendix Table B-1 for listings by specimen. Tissues sampled with culturette for general bacteriology consisted of brain, with liver and lung also occasionally sampled. A random sample of 24 virology samples was examined by electron microscopy for influenza or other viruses (reovirus).

Description	S. Puget Sound	Hood Canal	North of Puget Sound	All
General Bacteriology				
Number sampled	22	7	21	50
E. coli	2	0	11	13
Corynebacterium sp.	1	0	0	1
Pasteurella sp.	1	0	0	1
b-streptococcus sp.	2	0	2	4
a-streptococcus sp.	1	0	0	1
Acinetobacter sp.	2	0	0	2
Proteus sp.	2	3	10	15
Pseudomonas sp.	1	0	0	1
Enterobacter sp.	1	0	0	1
Virology				
Number sampled	24	5	20	49
San Miguel Sea Lion Virus	0	0	0	0
Influenza-like virus	1	0	3	4
Reo-like virus	0	0	1	1
Leptospira				
Number sampled	10	3	10	23
Leptospira found	0	0	0	0

regions. Samples from southern Puget Sound had a much wider variety of bacteria found than animals from Hood Canal or north of Puget Sound; five bacteria were found in southern Puget Sound that were not seen from either of the other areas.

Leptospira and San Miguel Sea Lion Virus (SMSV) were not recovered from any of our samples. Both these pathogens were tested for after we started observing the high proportion of premature births that occurred on Smith Island. Unfortunately few premature pups were recovered after we began sampling for Leptospira and SMSV. We cannot therefore rule out the possibility that either of these disease agents may have played a role in the premature births observed at Smith Island as has been described for premature births in other pinnipeds (Gilmartin et al., 1976; Smith et al., 1974a, 1974b).

Virions with morphologies typical of influenza viruses and reoviruses were seen from 24 randomly selected virology samples. Four samples with influenza virus (one pup in Eld Inlet, three from Smith Island), and one with reovirus (pup from Smith Island) were seen.

Influenza and a reovirus were identified in dead pups on Smith Island, and could have played a role in premature pupping. Although our virology sample of the premature pups at Smith Island was small, the high rate of premature pupping we found may be related to viruses. Premature pupping in California sea lions has been linked to two disease agents including a virus indistinguishable from Vesicular Exanthema of Swine virus (Gilmartin et al., 1976; Smith and Akers, 1976). Huber et al. (1984) report a high rate of premature pupping in northern sea lions on the Farallon Islands, California. Seven of ten sea lion pups born on the Farallons in 1983 were premature and died. Influenza virus was seen in five of these animals. The concurrence of influenza virus from a number of animals and high rates of premature pupping in northern sea lions on the Farallons and harbor seals on Smith Island, suggests influenza virus may contribute to premature pupping.

Influenza virus has recently been implicated as the cause of a mass mortality of harbor seals in New England (Geraci et al., 1982). Over 400 harbor seals died in 1979 and 1980 from pneumonia associated with this virus. Geraci et al. (1982) conclude influenza viruses may have been responsible for numerous historical mass strandings and mortality in pinnipeds.

Many of the bacteria we identified from harbor seals have been found in other pinnipeds, these include E. coli, Alpha-streptococcus sp., Betastreptococcus sp., Acinetobacter sp., Proteus sp., and Pseudomonas sp. (Baker and Doidge, 1984; Reijnders et al., 1981; Anderson et al., 1979; Baker et al., 1980; Bonner, 1970; Medway, 1980; Geraci et al., 1982). The role of specific bacteria in any of the causes of death we found is difficult to determine because of the lack of a consistent pattern. Geraci et al. (1982) concluded the bacteria isolated from seals apparently dying from the influenza virus were not a contributing cause of death because there was not a consistent pattern in the bacteria found. Corynebacterium sp. was isolated from the infected eye of a live pup at Smith Island; Bonner (1970) also reported isolating a Corynebacterium sp. from the infected eye of grey seal.

Disease may have played a role in the starvation of harbor seal pups. Starvation in neonates is generally considered to be the result of mother/pup separation (Le Boeuf et al., 1972; Bonner, 1975; Johnson, 1977). Observation of northern fur seal pups from birth until their eventual death from starvation indicated separation of mother and pup is often not the cause of starvation (Calambokidis and Gentry, 1985). Observations of weak lone pups on the beach at Smith Island prior to their becoming emaciated suggests these animals were sick from something other than starvation. The influenza virus that was seen in three pups from Smith Island or some of the bacteria found in dead seals may have served to debilitate the mother or the pup. Disease agents may have caused agalactia (reduction of milk production) in the mother, anorexia (failure to eat) in the pup, or reduced the ability of the pup to stay with its mother. The Smith Island pup that was found to have reovirus, was extremely emaciated yet had fresh milk in its stomach. Bacterial infections have been implicated in causing agalactia in domestic animals (Ross et al., 1969). Viruses such as vesicular exanthema of swine that have been isolated from pinnipeds (Smith et al., 1974b; Sawyer, 1976) have also been shown to cause agalactia (White, 1940; Bankowski, 1965) and anorexia (White, 1940) in swine.

3.2.5 Adult and subadult mortality

We relied on the stranding network and other contacts to recover most of the adult and subadult seals that we examined. The number of dead animals recovered was highest in southern Puget Sound compared to the Hood Canal and north of Puget Sound. The higher recovery in southern Puget Sound is expected because this area is more densely populated and therefore presents a greater chance that dead animals will be observed. Often these animals were somewhat decomposed.

Table 12 reports the causes of death of the adult and subadult harbor seals that we examined between 5 October 1983 and 29 January 1985. Most of the animals recovered had signs of trauma, usually from a blow to the head. The number of animals where the cause of death is listed as unsuitable for autopsy includes skeletal remains. We examined 4 adult females from southern Puget Sound and 5 from the Hood Canal. No reproductive abnormalities were observed. A high percentage of adults recovered were female from southern Puget Sound and the Hood Canal; and six out of nine females were recovered during or soon after the pupping season and were pregnant or post-partum. Adult females, near the time of parturition, may be more susceptible to human-related mortality (i.e. being hit by a boat).

Histopathology examination was limited to fresh animals. Cause of death information collected by animal is listed in Appendix Table A-12. The only adult animal observed with gross abnormalities was a male (CRC-215) from Whidbey Island. It had been recovered after an oil spill in Puget Sound in December 1984. This animal had severe pneumonia and was emaciated. Inflammation and a skin ulcer were found on a pregnant female (CRC-117) recovered from Budd Inlet in southern Puget Sound. We found no obvious cause of death.

Other research on harbor seal mortality is consistent with our results, finding that human-related deaths are the major cause of mortality in Oregon and Washington in the 1970s (Stroud and Roffe, 1979; Johnson and

Table 12. Cause of death of adult and subadult harbor seals recovered between 5 October 1983 and 29 January 1985 by region: southern Puget Sound (SPS), Hood Canal (HC), and north of Puget Sound (NPS).

Cause of death	SPS	HC	NPS	All sites
Blow to head	2	4	-	6
Shot	1	1	-	2
Other trauma	1	-	-	1
Incidental catch-fisheries	1	-	-	1
Drowning*	2	-	-	2
Suspected infectious agent	-	-	1	1
Not determined or unsuitable	11	3	2	16
Total	18	8	3	29

* Does not include animals that drowned as a result of other injuries included in another category (i.e. trauma).

Jeffries, 1977). Both of these studies, however, found a higher rate of gunshot deaths than we found.

3.2.6 Size class frequency and population structure

Figure 6 shows the differences in the size distribution of seals for the four sites that had over 50 seals measured. We found significant differences between sites in the proportion of seals in different size classes. The two sites north of Puget Sound, Protection and Smith Islands on 13 July, were not different from each other. All three regions (combining Smith and Protection Island data) had significant differences in size class distribution from each other (chi-square, $p<.001$). We found no significant differences (chi-square, $p>.05$) between seals measured on different days (and months) at the Skokomish Delta and Gertrude Island sites (see Figure 7 for Gertrude Island comparison), however measurements made on 13 and 15 July were significantly different than those made on 24 August and 13 September ($p<.05$) at Smith Island. This difference may in part reflect temporal changes of behavior of different age classes.

Of some interest is the high proportion of seals in the 1.2 to 1.3 m class for the areas north of Puget Sound. Through interpolation between adult reproductive female sizes and pup sizes and comparison with data from collected animals (Bigg, 1969a), this size class appears to represent primarily 2 and 3 year olds. Several explanations exist for this high proportion: 1) a boom year for pup survival occurred 2 or 3 years ago, 2) some hauling behavior is not uniform among age classes, or 3) some yet undiscovered bias has occurred in the photogrammetric measurements.

Based on photogrammetric measurements, not only were seals larger at Gertrude Island than at Smith Island, but pups with females were significantly longer at Gertrude Island than Smith Island (t-test, $p<.02$). The mean size of females was also larger at Gertrude Island compared to Smith Island but the difference was not statistically significant (Gertrude=1.45, n=17; Smith=1.39 m, n=21; t-test, $p>.05$). Mean length of dead pups recovered at Gertrude was longer than that for those at Smith, though the difference was again not statistically significant (84.1 cm, n=12, and 81.6 cm, n=21, respectively, t-test, $p>.1$). The standard length of adults recovered or collected since 1975 also tends to indicate seals from southern Puget Sound appear to be longer. Four of 10 adults stranded in southern Puget Sound had a standard length of greater than or equal to 1.65 m, while only one of nine adults from north of Puget Sound or from the Hood Canal were greater than or equal to 1.65 (stranding data 1975-1984 compiled from Johnson and Jeffries (1977), Calambokidis et al. (1978), and this study). None of 17 adults (at least 5 years old) collected in Grays Harbor were longer than 1.65 m (Johnson and Jeffries, 1983). The only animals longer than or equal to 1.70 m were two from southern Puget Sound.

Several possible explanations exist for significant differences in sizes of animals by site. Differences in size could signify genetic differences between regions. Section 3.2.7 discusses the evidence for genetic differences based on differences in pelage. Larger sized seals may also result from a population not yet at equilibrium with the environment. Fowler (1984a) reviews the evidence for density dependent body size in northern fur seals and concludes that body growth shows density dependent relationships. If Gertrude Island seals were further below carrying

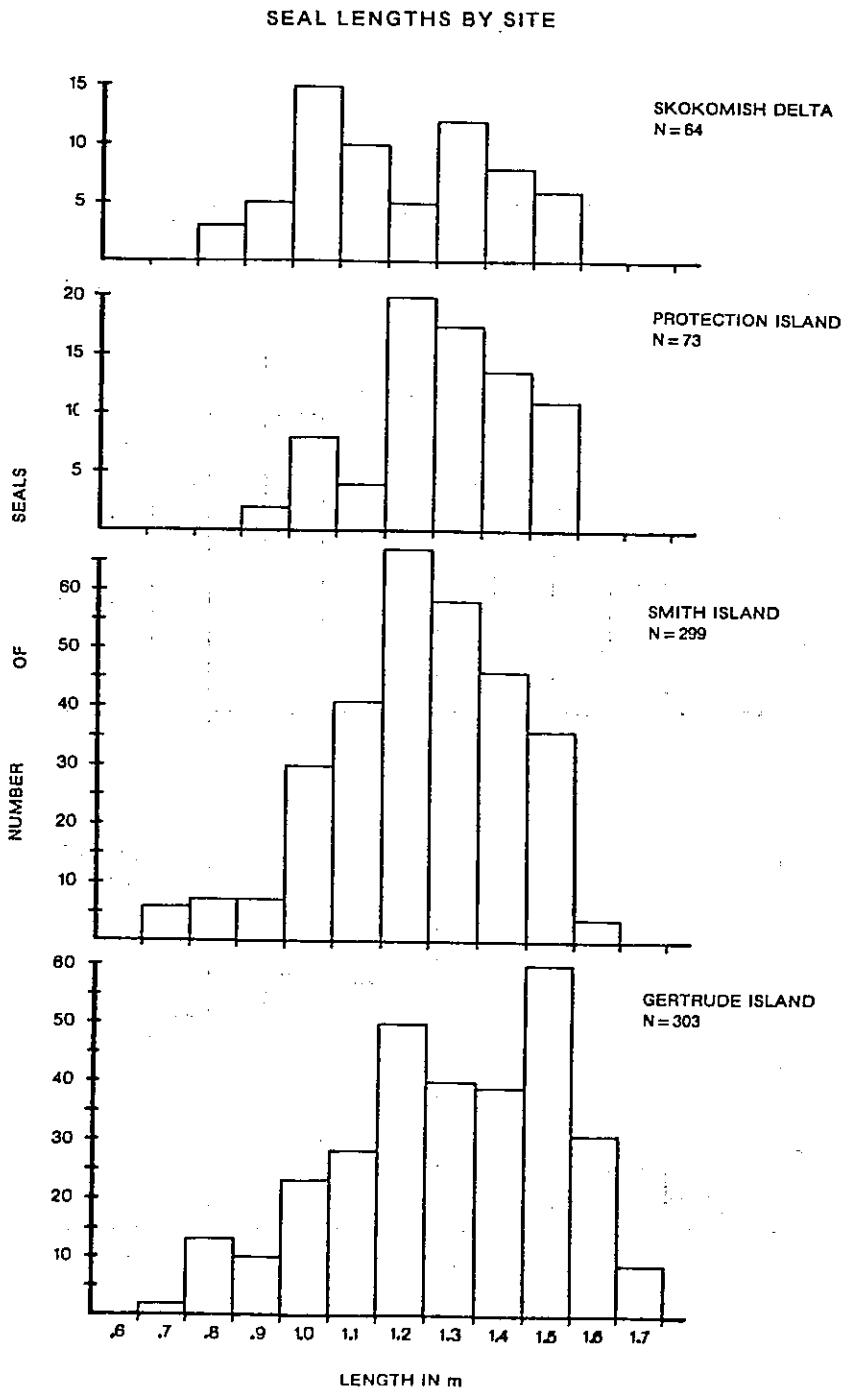

Figure 6. Size distribution of harbor seals measured through photogrammetry at four study sites in western Washington in 1984. Measurements were from more than one day for all sites (Table 4).

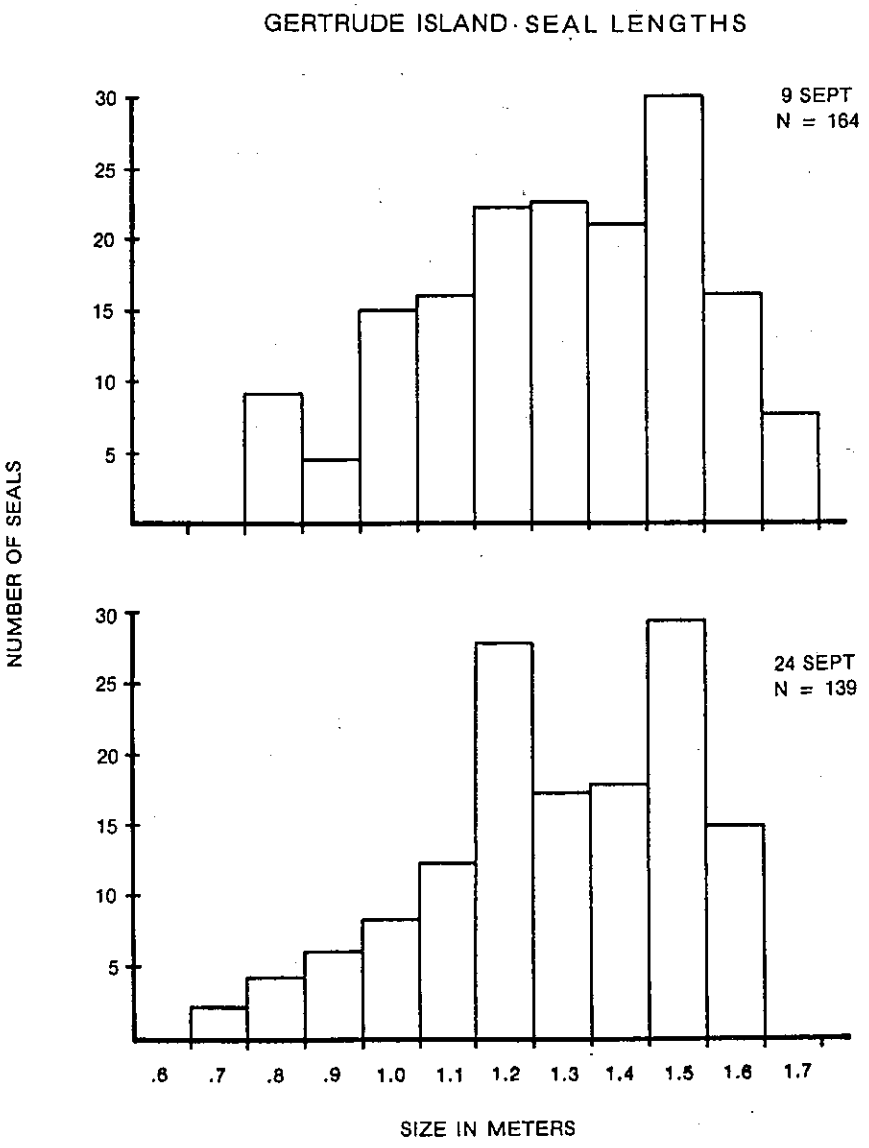

Figure 7. Size distribution of harbor seals at Gertrude Island measured through photogrammetry on two days in 1984.

capacity than other areas, then an increased size of individuals compared with other areas might be expected.

3.2.7 Pelage patterns

Pelage types of 1,095 seal sightings were tallied throughout the study to examine the presence of any pelage abnormalities or anomolies in our target areas. The proportion of seals by site for each of five pelage categories is shown in Figure 8. Most surprising in these results is the high proportion of seals showing an indistinct pelage. This type of pelage has not been described by other researchers except during the annual molt. Most of our sampling was conducted during times of year when seals were not molting, so we considered the indistinct pelage type as an anomaly. We have not had a chance to examine a stranded animal with this pelage type yet and so can only speculate on the cause of this type of pelage.

The most common pelage type at all sites was the light pelage, with 74% of the seals observed showing this basic pelage type. Seals with the indistinct pelage were the next most numerous at 21%. Dark pelaged seals were third most common at 3.8%. The two other pelage types (mixed and reddish) were encountered less than 1% of the time. There were clear differences in the proportion of several pelage types by site.

Indistinct pelage. There were no significant differences in the percentage of indistinct pelaged seals at the reference sites in the Hood Canal and San Juan Islands, so these sites were pooled. Indistinct pelage occurred significantly more frequently (chi-square, $p<.001$) in southern Puget Sound (23%, n=963) than in the Hood Canal and the San Juan Islands area (4.4%, n=316)(chi-square, $p>.05$). The proportion of indistinct pelage varied significantly between the three sites in southern Puget Sound (chi-square, $p<.001$). Indistinct pelage occurred in 27% of the seals sampled at Gertrude Island (n=751) compared to 8% of the seals sampled at Henderson Inlet (n=188), and 13% of the seals sampled at Budd Inlet (n=23). There was a higher proportion of seals with indistinct pelages at the two southern Puget Sound sites besides Gertrude Island than in our reference sites, but this difference was no longer significantly different (chi-square, $p>.05$). The primary reason for the significant differences in indistinct pelaged seals in southern Puget Sound therefore was the pelage of Gertrude Island seals.

To further examine this pelage abnormality we tested for differences in indistinct pelage by sex and age class. Figure 9 shows the proportion of different pelage types by sex and age-class of seals (all sites included). Indistinct pelage varied significantly by age class (chi-square, $p<.001$) with subadults showing a much higher incidence of this pelage type than adults or pups. Males also tended to have a higher proportion of indistinct pelage than females (chi-square, $p<.01$) though this difference was not as dramatic as the differences between age classes. Subadult males had the highest proportion of indistinct pelage at 42%. For Gertrude Island seals, 54% of the subadult males showed the indistinct pelage.

The differences in indistinct pelage by age class suggests seals develop this condition as subadults and then grow out of it before becoming adults. During the molt at Gertrude Island we did observe several

Figure 8. Harbor seal pelage type by site. Detailed pelage descriptions in Section 3.1.8.

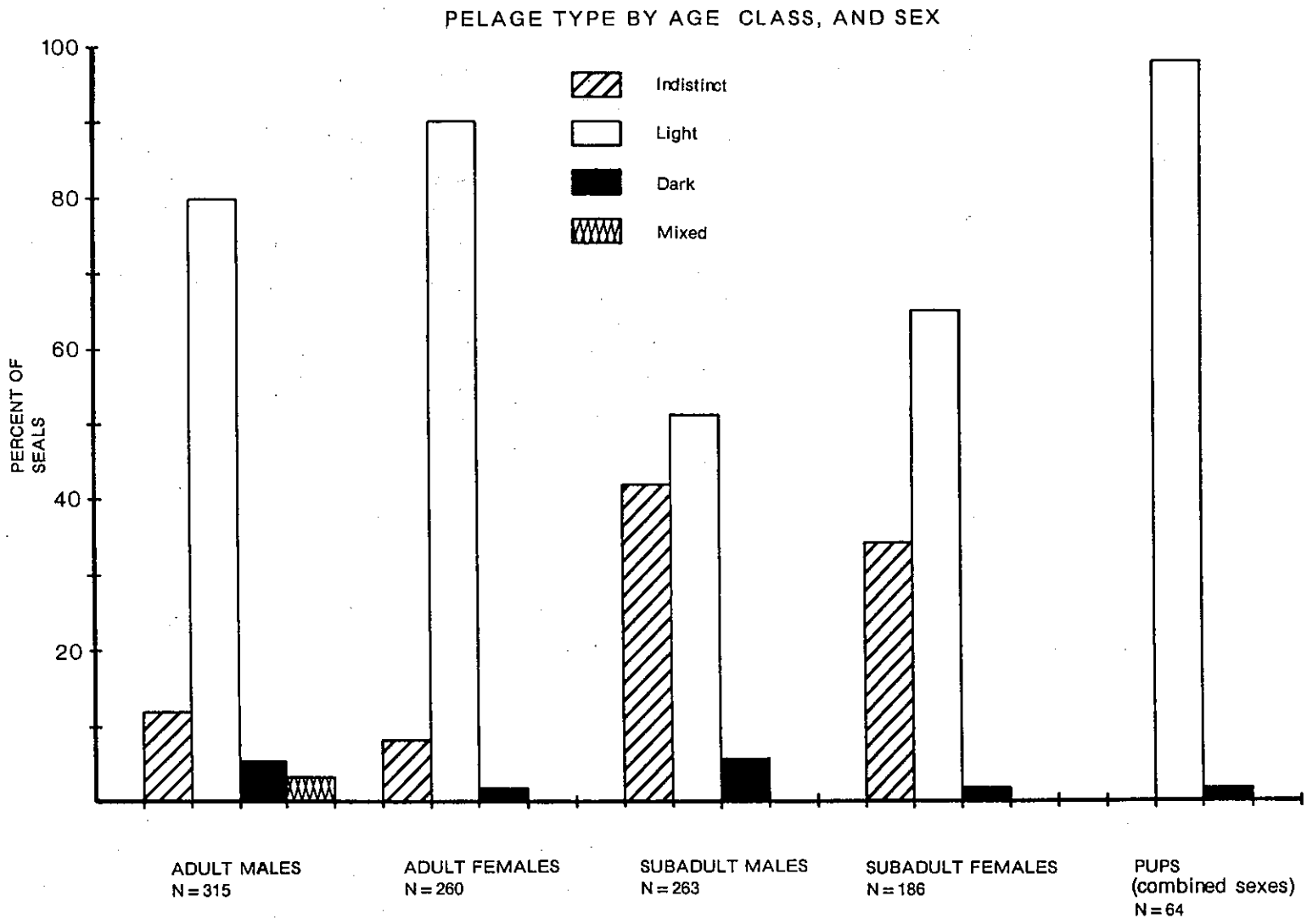

Figure 9. Pelage type of harbor seals at all sites by age class and sex. See Section 3.1.8 for description of pelage types.

subadults with indistinct pelage molting out of this pelage into a standard pelage. The molt in these individuals was different than in other seals. The old indistinct pelage appeared to fall away on sections of the body leaving bare skin before the new silver and black pelage set in.

Stutz (1967) reported differences in the light and dark pelage patterns in harbor seals from different sites in British Columbia and Alaska. He separated pelages of harbor seals into three categories; common, dark, and muddy. The first two categories closely resemble the terminology and definitions we used. The muddy pelage also seemed similar to our indistinct pelage though it is difficult to evaluate the similarity between Stutz's descriptions and our own. This is complicated by Stutz's use of photographs of coats that had already been skinned out and our use of field observations. Shaughnessy and Fay (1977) reported on the clinal variation of the dark and light color morphs in harbor seals along the N. Pacific. They suspected that the muddy coat described by Stutz may be from animals in the molt and does not represent a distinct morph. Our findings also suggest that the indistinct pelage does not represent a true morph. The indistinct pelage type does not appear to be normal because of its low frequency at all sites except Gertrude Island. The similarity of the indistinct pelage to that of molting seals suggests this pelage may be related to an abnormality in the normal molt.

Dark and mixed pelage. These two types of pelage were fairly similar and might even be considered as one type. As with indistinct pelage there were regional differences (Figure 8). The reference sites in the Hood Canal and San Juan Islands were again not significantly different (chi-square, $p>.05$) and were pooled. Seals in southern Puget Sound had a significantly smaller incidence of dark pelage than the reference areas (chi-square, $p<.001$). This difference did not seem to vary between the different sites in southern Puget Sound, though this was difficult to test do to the low incidence of dark pelage in all areas. The incidences of mixed pelage followed similar site patterns as the dark pelage.

Unlike indistinct pelage, the incidence of dark pelage did not vary by age-class (chi-square, $p>.05$). There was also a significant difference between males and females in the incidence of dark pelage (chi-square, $p<.001$). This difference, however, may be the result of the greater number of males than females that were sampled in the reference areas, the area which also had the higher proportion of dark pelage seals. We could not find a significant difference between males and females when we tested the reference areas and southern Puget Sound separately (chi-square, $p>.05$).

Stutz (1967) suspected differences in the proportion of dark-pelaged harbor seal were related to relative genetic isolation of some of these populations. Shaughnessy and Fay (1977) reported the proportion of dark morphs was highest in Baja, at the southern end of the harbor seal's range, decreased to a low in the Prince William Sound area, and increased again through the Aleutians to Hokkaido, Japan. This pattern parallels changes in body size of harbor seals that were largest in Baja, Mexico and Japan and smallest in Prince William Sound, Alaska (Burns and Golt'sev, reported in Kelly, 1981). Kelly (1981) found that the ratio of light phase to dark phase in pups with light mothers showed a good fit with expected values predicted by the hypothesis that the pelage dimorphism is controlled by a pair of autosomal alleles with light phase dominant over black. The pups

born to dark mothers, however, did not match the expected values for this hypothesis. Kelly (1981) found no evidence of sex differences in pelage type, though Scheffer and Slipp (1944) reported males tending to be darker than females. Our results were ambiguous on this question.

3.2.8 Umbilical lesions

We found a consistent pattern of scarring and lesions around the umbilicus in a number of harbor seals. The scarring varied in severity from appearing to be a slightly enlarged umbilicus, to a 20-30 cm diameter open lesion or ulcer around the umbilicus. The lesion had often appeared to have healed over but had left a scar around the umbilicus. We did not find this scarring in any of the stranded animals. The incidence of these lesions at a number of sites throughout our study regions was tallied during scans of seals to determine sex, age classes, and pelage patterns of seals.

The incidence of umbilical scarring or lesions varied significantly by region (Figure 10). Out of 194 animals observed at a close enough distance to determine the presence of lesions in the Hood Canal and San Juan Islands, only one animal was seen with even slight indications of the scarring or lesions described above. In southern Puget Sound, however, 125 out of 901 (14%) animals sampled had either scarring or ulceration around the umbilicus. There were also significant differences between our three sites in southern Puget Sound (chi-square, $p<.001$). The frequency of scarring or ulceration of the umbilicus was 17% at Gertrude (n=752), 2% at Henderson Inlet (n=126), and 0% at Budd Inlet (n=23).

The incidence of these lesions varied significantly by age class (chi-square, $p<.001$) with lesions occurring in subadults more than twice as frequently as in adults. Lesions were not seen in pups, though on four occasions we observed what may have been the early stages of these lesions in post-weaned pups. No difference was found between males and females (chi-square, $p>.05$).

There appeared to be an association between umbilical lesions and the indistinct pelage discussed earlier. Both these disorders occurred most frequently in subadults at Gertrude Island. Even within this subgroup, however, subadults at Gertrude with indistinct pelage had a significantly higher rate of umbilical lesions than Gertrude subadults with other pelage types (chi-square, $p<.001$); 37% of those with indistinct pelage had lesions versus 16% of those with other pelage types.

Skin lesions have been reported to occur in harbor seals in the Wadden Sea (Drescher, 1978; Reijnders et al., 1981) as well as in grey seals in Britain (Anderson et al., 1974). The skin lesions reported in the Wadden Sea harbor seals occurred in 40% of the juvenile seals in some areas (Drescher, 1978). Reijnders (1980, 1981) reported that this high incidence may partly be the result of the immuno-suppressive effects of the high PCB concentrations found in these seals. The appearance and locations of the lesions found in southern Puget Sound harbor seals is identical to those seen in the Wadden Sea (Reijnders, pers. comm. in response to photographs of the southern Puget Sound lesions). The infection is suspected to be the result of a disease agent and mechanical injury caused by sand substrate (Reijnders et al., 1981). The frequent disturbance that seals in the

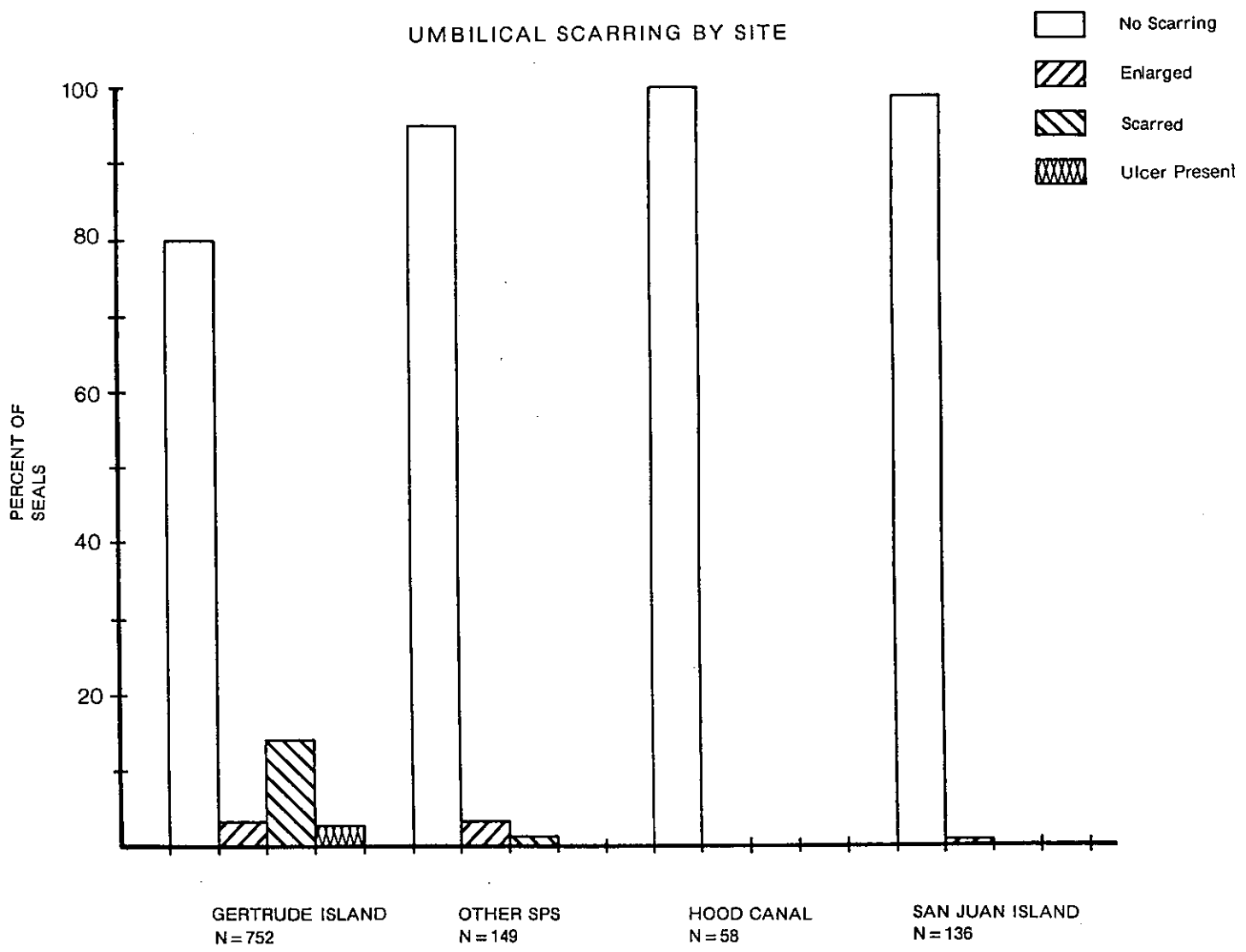

Figure 10. Proportion of harbor seals with umbilical lesions by site. See Section 3.1.8 for description. Other SPS refers to observations at our other study sites in southern Puget Sound at Budd and Henderson Inlets.

Wadden Sea are subjected to was also thought to be partly responsible for the failure of these lesions to heal. Gertrude Island seals also haul out on a sand substrate and, as reported in the Wadden Sea, the umbilical lesions may be related to the combined effect of sand abrasion and contaminant exposure.

3.2.9 Harbor seal population model

In order to test the internal consistency of our figures on increases in seal numbers, reproduction, and mortality, we constructed a Leslie-matrix population model for harbor seals. Southern Puget Sound was chosen as the region to test because: 1) it is the target study area and of greatest concern, 2) the harbor seal population appears to be distinct from neighboring seal groups with little apparent emigration and immigration, 3) we have good census coverage for all haul-out areas in this region, and 4) the high human population density in this area would result in the highest chance of dead seal reports and recovery. The model incorporated and tested the degree of agreement between our measurements of population increases, reproductive rate, neonatal mortality, adult mortality, and size distribution.

Input parameters required for the model were the reproductive rate (as a percent of the non-pup population), the neonatal mortality rate (to year 1), and the adult mortality rate. Outputs after the modeled population was allowed to stabilize were annual rate of increase, percentage of adult females that would need to be pregnant for the given population structure and reproductive rate, and percentage of the population that were adults (> 4 years old). One assumption of the model was that the maximum possible age for a seal was 25 years. The model was not very sensitive to this assumption because at the high rates of population growth we were modeling, the older animals become a fairly insignificant proportion of the population.

Initial input parameters were derived from observed rates reported in earlier sections:

1) The reproductive rate is 25% of the pre-pupping population.

Based on southern Puget Sound figures in Table 7.

2) Survival of seals to the first year is 70% of those born (full-term or premature).

Derived from the total pup mortality of 20% reported for southern Puget Sound in Table 9. We assumed that 2/3 of the seals that died in southern Puget Sound were found during our searches or reported to us through the stranding network. Dead seals that were not recovered immediately were frequently reported to us by several people especially when the carcass was afloat and had washed up at several locations. From these subjective observations we concluded that most of the seals that die in southern Puget Sound are reported, and that our 2/3 estimate is reasonable.

3) Annual survival from 1 to 25 years of age is .97.

This estimate is based on adult and subadult mortality in southern Puget Sound reported in Table 12 and Appendix Table A-12 taken as a percentage of the estimated population of 700 harbor seals in southern Puget Sound in 1984. We have again employed our estimate that we recover 2/3 of the stranded animals as discussed above. Figures in Table 12 were adjusted to exclude animals that did not die in 1984.

The output from this model after it was allowed to stabilize agreed very well with other independent measurements of the population. The calculated annual population growth rate was 14% per year which generally matches the average of the growth rates for the four study sites in southern Puget Sound (Section 3.2.2). From this model 83% of females 4 years old or older would need be pregnant. This is in close agreement with our observation of 82% of adult females pregnant or with pups at the start of the pupping season at Gertrude Island (Table 8). Finally, the model predicts that at the values used, 61% of the population (not including pups) would be 4 years old or older. This matches our photogrammetric calculation of 62% adults at Gertrude Island.

The close agreement between this model (using observed birth and mortality rates) and the independently calculated population growth rate, pregnancy rate, and general age structure for the same location strengthens the reliability of our measurements of all these parameters and supports the accuracy of the independent measurements.

3.3 Conclusions

The effects of pollution on harbor seals could not be monitored in the areas of highest pollution concentrations (central Puget Sound) because of the absence of seal haul-out sites in these areas. This absence may reflect an historical or current adverse effect of human activities on seals in these areas. Harbor seals in southern Puget Sound, which would be expected to have the highest pollutant concentrations, appear to be doing well. Population growth rates, reproductive rates, and mortality rates all seem as good or better than for our reference sites north of Puget Sound and in the Hood Canal. Information from the 1970s suggests the growth in numbers of harbor seals was low in southern Puget Sound compared to those in other parts of the state. The continued population growth rate of seals in areas north of Puget Sound and Hood Canal may be bringing these populations closer to carrying capacity. The apparent low rates of increase in southern Puget Sound compared to other parts of the state in the mid 1970s may have allowed the southern Puget Sound harbor seals to increase faster in the late 1970s and early 1980s because the population was further below carrying capacity.

We did not find any evidence of high rates of premature births, neonatal deaths, and reproductive failure in the southern Puget Sound seals as has been associated with pollutants in pinnipeds from other parts of the world (Gilmartin et al., 1976; Helle et al., 1976a; Reijnders, 1981). We did find surprisingly high numbers of premature births and neonatal mortality in harbor seals at Smith Island, north of Puget Sound. This high

mortality appears to be linked to disease agents, possibly an influenza virus. This high mortality may also indicate that seals in these areas may be approaching the carrying capacity. As the population approaches carrying capacity density-dependent neonatal mortality, documented in other pinnipeds (York, 1985; Fowler, 1984b; Summers et al., 1975; Doidge et al., 1984), would be expected to increase or become more highly variable. Verification of the consistency of the patterns we noted over more than one year is needed to test these hypotheses.

Harbor seals in southern Puget Sound appear to be distinct from populations in Hood Canal and areas north of Puget Sound. Seals in southern Puget Sound have a different proportion of a genetically controlled pelage pattern, have different pupping seasons, and have a different size distribution than seals from neighboring areas. The isolation of seals in southern Puget Sound would be facilitated by the large buffer zone in central Puget Sound that is occupied by only a small number of seals. The larger maximum lengths of seals in southern Puget Sound may be the result of other factors besides genetic differences. Differences in food habits or a density dependent growth rate could also account for differences developing between seals in different regions.

The high incidence of a pelage anomaly and umbilical lesions in southern Puget Sound could be related to higher contaminant concentrations in these areas compounded by sand abrasion. Indistinct pelage patterns and skin lesions are also seen in harbor seals in the Wadden Sea (Reijnders, pers. comm.), an area with high contaminant concentrations similar to those in parts of southern Puget Sound.

Knowledge of current contaminant concentrations in harbor seals would be extremely helpful to: 1) test assumptions of population differences relative to contaminant concentrations, 2) test for evidence of increase or decrease in contaminant levels in seals over time, 3) provide comparisons of our findings with those in other areas where disorders have been linked to contaminants, and 4) allow us to test hypotheses regarding specific correlations between some of the pathology and microbiology we found in individual animals and their contaminant burdens.

4. OTHER MARINE MAMMALS

Though harbor seals were the primary study species, additional research was also conducted on killer whales (Orcinus orca), harbor porpoise (Phocoena phocoena), and river otter (Lutra canadensis). The type of data and the study approach differed for each animal. During the course of this study data were also gathered on other species of marine mammals including the gray whale (Eschrichtius robustus), California sea lion (Zalophus californianus), and northern sea lion (Eumetopias jubatus). Data gathered on these species did not follow the same study design as research reported here, and will be contained in manuscripts currently in preparation.

4.1 Killer Whale

High levels of PCBs and DDT (250 ppm and 640 ppm, respectively) have been found in the blubber of a killer whale in Washington State (Calambokidis et al., 1984). Because of their position high on the food chain, killer whales are likely to be exposed to high concentrations of bioaccumulated contaminants through their prey. To test for possible effects of contaminants in killer whales, we compared measurements of reproductive success and mortality between groups of killer whales likely to be exposed to different levels of contaminants.

Killer whales live and breed in the waters of Washington State (Osborne et al., 1985; Bigg, 1982), in discrete pods of individuals that appear to remain stable from year to year (Bigg, 1982; Balcomb et al., 1980). Three major groups, or, communities of these pods--southern resident pods, northern resident pods, and transient pods--have been identified in the waters off Washington and Vancouver Island, Canada (Bigg, 1982; Osborne et al., 1985). These different communities are defined primarily by their locations and movements, and possibly, foraging behavior (Osborne et al., 1985). The southern and northern resident communities of pods have separate ranges that border on the tidal boundary half-way up the east side of Vancouver Island. The transient community pods occur throughout both resident pod ranges and appear to vary to a greater extent than the resident pods from the the usual diet of salmon and feed on marine mammals (Osborne et al., 1985).

We compared population parameters of gross recruitment, mortality, and net recruitment between the three communities of pods as coarse tests of the occurrence of biological disorders in killer whales in relation to the expected levels of contaminants. These tests relied on some assumptions: 1) the northern community pods have the lowest overall level of contaminants as they feed off comparatively pristine and non-industrial areas of northern Vancouver Island; 2) the southern community pods have higher levels because of their proximity to industrialized areas of Tacoma, Seattle and Vancouver, British Columbia; and 3) the transient pods have the comparatively highest levels because it appears that their diet is made up of a greater proportion of marine mammals than that of the other two communities of pods and would thus be feeding at a higher trophic level (Osborne et al., 1985).

4.1.1 Methods

Our estimates of population parameters for killer whales (total population, population changes by year, and number of births and deaths by year) between 1975 and 1985 were based on studies by Bigg (1982) and Balcomb et al. (1980), as well as a recent data synthesis prepared by Moclips Cetological Society in Friday Harbor, Washington under subcontract from Cascadia (Osborne et al., 1985). These studies are based largely on boat and land-based sightings as well as photographic identification of individuals. Pod size, community size, numbers of cows, calves, births, and deaths were determined by year, by pod, and by community (northern, southern and transient). Values for similar time periods were compared when possible.

4.1.2 Results and Discussion

Figure 11 shows birthrates and mortality rates for communities where years of comparison are similar. Appendix Table A-13 shows the derivation of these rates. The data are incomplete and do not allow for comparisons between all three communities for all years. For different measures of mortality, natality, and net recruitment, where years are similar, the southern pods appear to have slightly lower population growth than the northern pods. However, more striking is the apparent population decline in the transient pods between 1974-1977. This decline is contrasted with an increase in the southern pods over the same period. The mortality in the southern pods has increased significantly (chi-square, $p<.05$) from periods 1973-1980 to 1981-1984. During 1981-1984 the southern pods also had a low birth rate and the population size decreased by 10%, though these data are still tentative.

Osborne et al. (1985) review available data and conclude that the transient pods appear to feed on marine mammals to a greater extent than the resident pods which feed on salmon and other fish. Transient pods were observed preying on marine mammals 4% of the time, whereas the southern residents were seen predating on marine mammals less than 1% of the time. The only stomach contents analysis of a transient community animal revealed only marine mammal remains (Bigg, pers. comm. in Osborne et al., 1985). One stomach of a resident community animal showed only salmon remains and no marine mammal parts.

Very high levels of PCBs and DDE were found in the blubber of the above mentioned adult transient male killer whale (250 ppm PCB and 640 ppm DDE; wet weight) and a resident male killer whale (38 ppm PCB and 59 ppm DDE; wet weight) (Calambokidis et al., 1984). The levels in the transient animal are much higher than any reported for all other examined marine mammals in Washington State (Calambokidis et al., 1984) and these high levels are consistent with the hypothesis that transient pods are feeding at higher trophic levels than the resident pods.

Several other differences besides feeding ecology exist between resident and transient killer whales. All pod types were selectively culled for immature animals in the 1960s and early 1970s for amusement parks and thus some population structure variations caused by this harvest could account for changes in population growth (Bigg, 1982). Other ecological considerations such as the comparatively smaller size of the

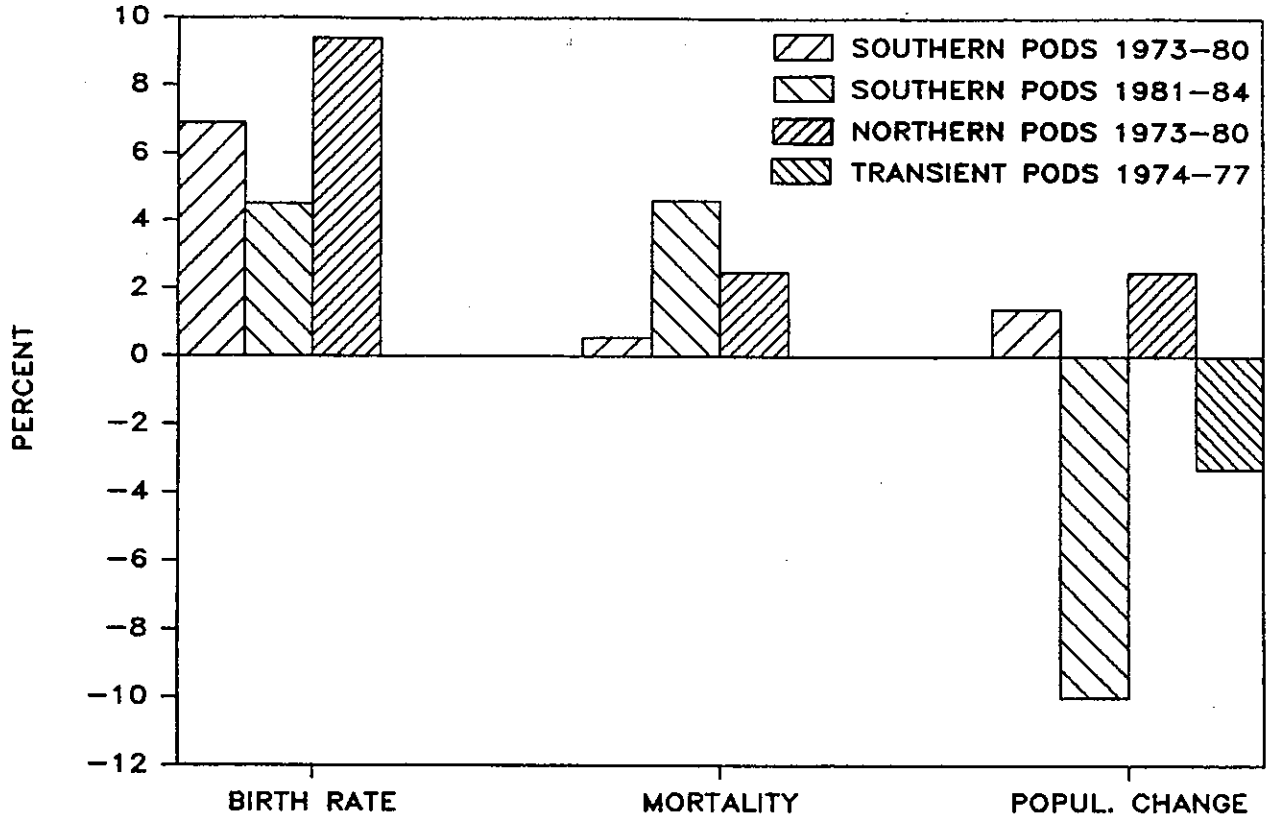

Figure 11. Comparison of three killer whale population parameters between different pod types.

transient pods (15 animals vs. 70 for the southern resident community) and the presumably greater migration distance of the transients also point to differences between the populations that may well be independent of contaminant differences. Still, the comparative decline of the transient pods is consistent with the hypothesis that these animals are suffering possible effects of higher levels of contaminants. The greater mortality, low birth rate, and apparent population decline of the southern resident pods in recent years is also consistent with the hypothesis of contaminant-related problems since the southern pods are probably exposed to higher levels of contaminants than the northern resident pods. More data are needed and may be available soon from other researchers on the current status of the northern resident pods and the transient pods. Certainly, if bioaccumulated contaminants present in Puget Sound can cause biological disorders, the killer whale, in view of its position high on the food chain, is at great risk.

4.2 Harbor Porpoise

We examined sightings of harbor porpoise to determine if this species remained absent from Puget Sound and still occurred in areas north of Puget Sound. Harbor porpoise populations have declined in a number of areas throughout the world and pollutants have been suspected as one of the possible causes (Otterlind, 1976; Wolff, 1981; Prescott and Fiorelli, 1980). High levels of PCB and DDT compounds have been documented in a harbor porpoise from southern Puget Sound (Calambokidis et al., 1984) as well as in harbor porpoise from the North Sea (Koeman et al., 1972), the Baltic Sea (Otterlind, 1976; Harms et al., 1978), off Rhode Island (Taruski et al., 1975), and off Southern California (O'Shea et al., 1980).

Though virtually absent from southern Puget Sound, our target study region, we recorded sightings of harbor porpoise when encountered in the course of our research on other species. Our primary objective was to evaluate the current distribution and general abundance of this species in and around Puget Sound.

We did not see and had no reports of harbor porpoise occurring in central or southern Puget Sound. All our harbor porpoise sightings were in areas north of Puget Sound. On two occasions, on 17 May and 24 June 1984, groups of 50 or more harbor porpoise were encountered in the northern San Juan Islands. The larger group contained an estimated 100 animals in loose groupings of two to about a dozen animals. Both these sightings and other sightings of smaller numbers occurred in the same general area just north of President's Channel in the San Juan Islands. One harbor porpoise was found dead on Bainbridge Island in Puget Sound.

Harbor porpoise concentrations of up to 100 animals as seen in the northern San Juan Islands are unusual for the San Juan Islands. Flaherty and Stark (1982) reported a much lower occurrence and smaller group sizes from their research on harbor porpoise in the San Juan Islands area. Groups of 100 have not been previously reported in this area (Everitt pers. comm.; Osborne pers. comm.).

Before the 1950s, harbor porpoise were considered the most common cetacean in Puget Sound (Scheffer and Slipp, 1948). Harbor porpoise once

were common in the Hood Canal where they were traditionally hunted by the Twanoh indians (Elmendorf, 1960). Our data on harbor porpoise in Puget Sound are consistent with other reports of their virtual disappearance from southern Puget Sound and Hood Canal in Washington State (Calambokidis et al., 1978; Everitt et al., 1979; Flaherty and Stark, 1982).

Population declines of the harbor porpoise in a number of areas have been suspected to be related to high concentrations of PCBs. Otterlind (1976) noted a drastic decline in the abundance of harbor porpoise along the Swedish west coast and in the Baltic Sea between 1940 and the mid-1970s and found high levels of both PCBs and DDT in the blubber of harbor porpoise from these areas in the 1970s. Otterlind (1976) concluded that the declines were most likely linked to high levels of PCBs. He also cited evidence that PCB concentrations were related to reproductive disorders found in seals from the Swedish west coast and the Baltic (Helle et al. 1976b), and suggested the possibility of a similar effect in harbor porpoise. Harbor porpoise have declined from most areas in the Wadden Sea and the declines are suspected to be linked to high levels of PCBs (Wolff, 1981).

Our results indicate that harbor porpoise continue to be virtually non-existent in the Puget Sound basin and the Hood Canal. The numbers of harbor porpoise in areas north of Puget Sound, however, are larger than reported in other recent research.

4.3 River Otter

The river otter frequents both freshwater and marine areas of Puget Sound and feeds on sculpins, flounders, crayfish, and during the fall, spawning salmon (Hirschi, 1978). The report by Henny et al. (1981) of possible links between river otter declines and PCBs in the Columbia River area led us to examine evidence for similar pollution related declines in Puget Sound.

To test for a parallel decline in Puget Sound, we examined the Washington State trapping records for evidence of river otter declines in industrial/marine areas as was reported by Henny et al. (1981) for the Columbia River, based on Oregon trapping records.

4.3.1 Methods

Since the number of otter trapped varies with effort, technique, fur prices, etc., we chose to analyze the data by computing the otter trapped in specific marine/industrial counties as a percent of total otters trapped in western Washington counties. We used only western Washington counties in our computation of the total otters trapped because of changes in management policy that have occurred in eastern Washington. We pooled the data into 5 year blocks for the period 1942-1983.

4.3.2 Results

The percent of otter trapped in the three counties we had identified as being the most marine/industrial (King, Pierce, and Kitsap) and that border Puget Sound showed no evidence of relative decline (Figure 12).

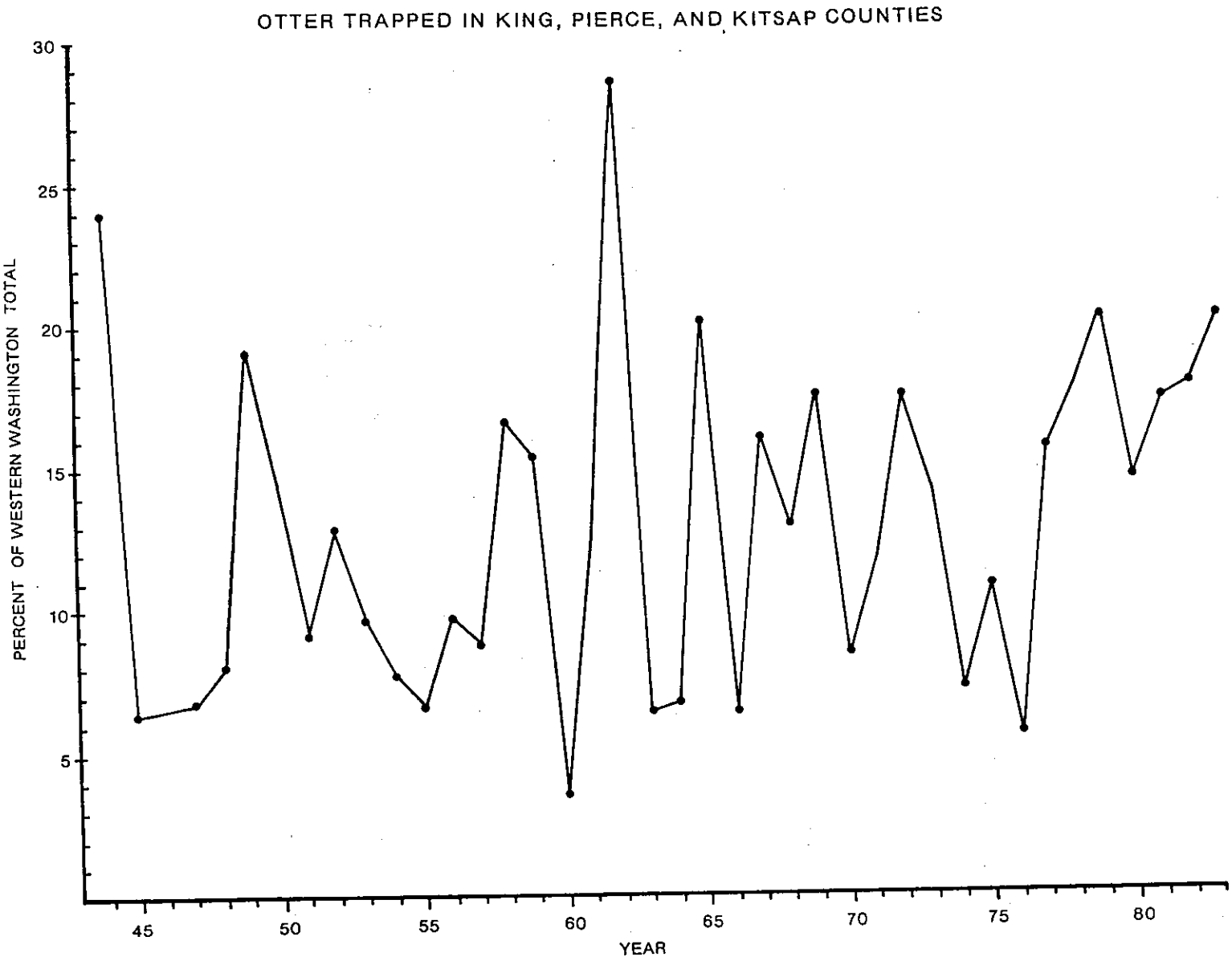

Figure 12. River otters trapped in King, Pierce, and Kitsap Counties as a percent of the total trapped in western Washington by year. From Washington Game Department records.

Otters trapped in these three counties in the 1978-1983 block comprised 18% of the western Washington total, higher than any previous block. For other 5 year blocks the percentage ranged between 10% and 15%. We therefore concluded that based on the trapping data, there was no evidence for a decline in river otter in the counties bordering Puget Sound. The method we used, however, is too crude to detect more local or subtle declines.

4.3.3 Discussion

PCB contamination has been associated with declines in otter populations from two areas. River otters collected in the lower Columbia River area of Oregon contained high levels of PCBs in liver and muscle tissue (a mean of 9.3 and 3.7 ppm, wet weight, respectively, in five males) (Henny et al., 1981). River otters collected in other areas of Oregon contained low residues of PCBs. Based on the analysis of trapper harvest statistics, Henny et al. (1981) found that the lower Columbia River area showed a decreasing harvest during the period 1949-1976, while the river otter harvest has increased over this time in other areas of the state. They noted that PCB residues in livers of some river otters from the lower Columbia River were higher than the residues detected in livers of mink that died of PCB dosage during experimental studies, and concluded that population declines of river otters in the lower Columbia River may have resulted from high concentrations of PCBs.

Almkvist (1982) reported that otter populations have decreased severely along the Baltic coasts of Sweden and Finland, and notes high levels of PCBs in otters from the Swedish coast. Otters from a stable population in northern Norway had PCB residues an order of magnitude lower than those found in the otters from the Baltic coast of Sweden.

We are aware of only one report of PCB and DDT levels in one river otter from Puget Sound (Calambokidis et al., 1984). Residues reported by Henny et al. (1981) are for different tissues than those reported for the single Puget Sound sample (liver and muscle vs. fat). The residues Henny et al. (1981) report do seem to be fairly high and therefore may be higher than those in river otter from this area.

Trapping data for the counties bordering Puget Sound do not indicate a decline in river otters in these areas. Residue data from river otters from the Puget Sound area are needed to allow comparison with data reported by Henny et al. (1981).

5. GLAUCOUS-WINGED GULL

The Glaucous-winged Gull is ubiquitous in Washington marine waters. It is found in nearly every marine habitat in all seasons. There are over 36,000 nesting Glaucous-winged Gulls in Washington marine areas (Speich and Wahl, 1985). Colonies are located throughout western Washington, including the waterfronts of the major urban-industrial areas such as Seattle, Tacoma and Shelton. Although many birds forage in upland urban areas and garbage dumps, the species is basically tied to marine food chains. The Glaucous-winged Gull is an attractive species to investigate for evidence of possible effects of environmental contaminants for several reasons: 1) it has a widespread breeding and foraging distribution in Washington marine areas; 2) it breeds and forages in areas of known high contamination; 3) it is abundant; 4) colonies are easily accessed; and 5) other studies on gulls are available for comparison.

Studies conducted in the Great Lakes during the early 1970s have documented a number of biological abnormalities that were associated with high contaminant residues in eggs and body tissues of the Herring Gull (Larus argentatus)(Gilbertson, 1974; Gilbertson and Hale, 1974; Teeple, 1977; Gilbertson and Fox, 1977; Fox et al., 1978). These biological abnormalities included low hatching and fledging success rates, eggshell thinning and breakage, abnormal nesting behavior, and high embryonic mortality. Embryos that failed to hatch had enlarged livers, growth retardation, subcutaneous edema, and elevated liver porphyrin levels (Gilbertson and Fox, 1977). Abnormalities identified in Herring Gulls from the Great Lakes were believed to be associated with the high levels of DDE and PCBs found in eggs and tissues. High levels of chlorinated hydrocarbon pollutants were also associated with the mortality of over 100 Ring-billed Gulls (Larus delawarensis) found dead throughout southern Ontario, Canada during the summers of 1969 and 1973 (Sileo et al., 1977).

The primary objectives of this study of the Glaucous-winged Gull were to determine the reproductive health of the breeding populations at several study sites and to examine the incidence of histopathology in individual birds. Study sites were chosen to represent populations exposed to major sources of urban-industrial contaminants (target areas), and populations in relatively pristine uncontaminated locations (reference areas). If there were recognizable and quantifiable effects from exposure to environmental pollutants then we would expect to see these effects in the birds nesting at the urban-industrial sites. Particular aspects of reproductive biology and physiology were chosen for study because they were measurable in the field or laboratory, were identified in other studies as sensitive to environmental pollution, or were fundamental indicators of population reproductive health.

5.1 Methods

5.1.1 Study site selection and locations

Six sites were studied (Figure 13): Two were reference sites, chosen for their relative remoteness from contaminated industrial areas. These were Goose Island in Grays Harbor, and Smith Island at the eastern end of the Strait of Juan de Fuca off Whidbey Island. Three sites were located in

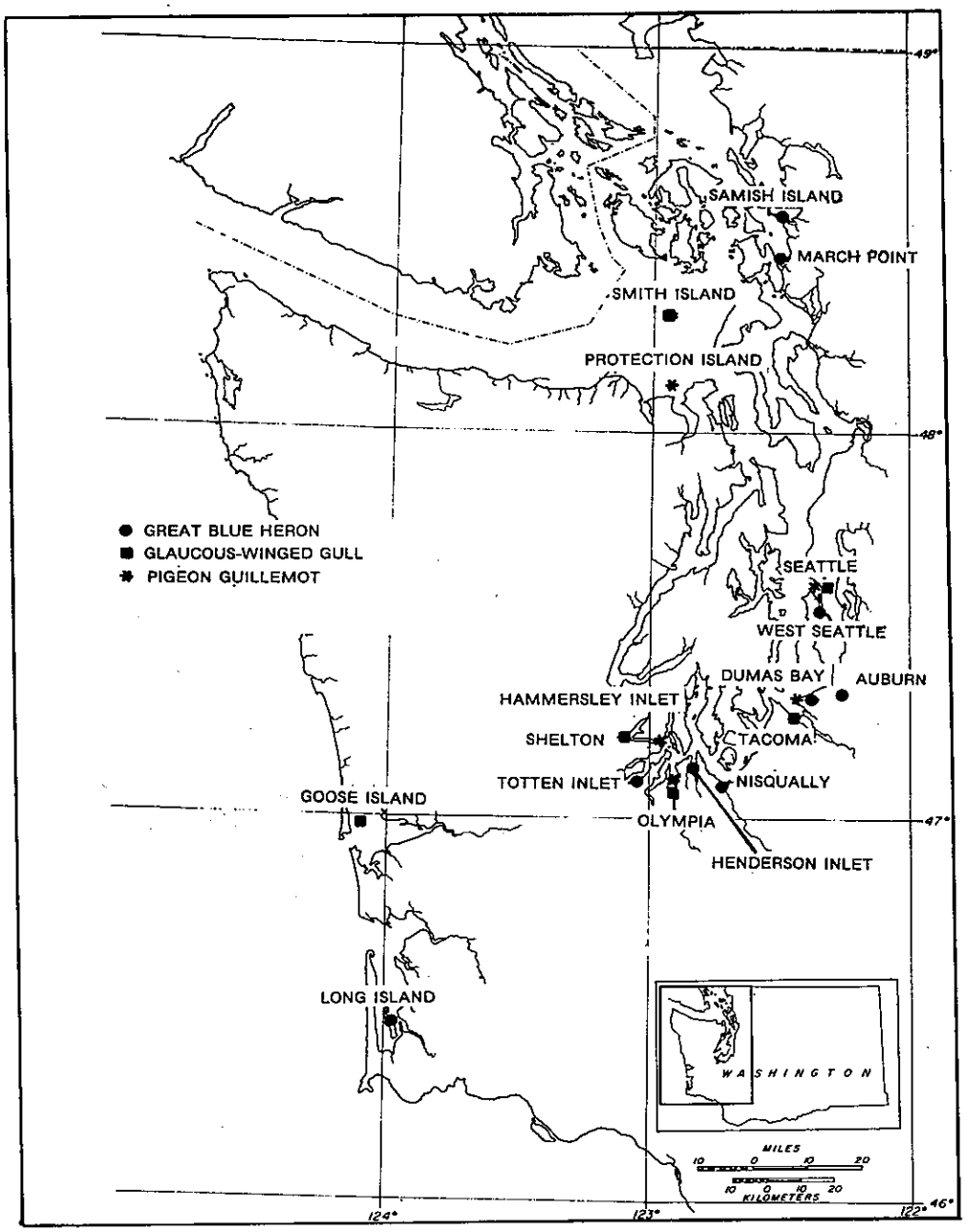

Figure 13. Marine bird study sites in western Washington, 1984.

industrial areas of relatively higher contamination. These target sites were Smith Cove at the north end of the Seattle waterfront, the St. Regis Paper Co. on the Tacoma waterfront, and the Simpson Timber Co. on the Shelton waterfront. The exact sites were chosen for the presence of nesting gulls and the ease of access to the colonies. The sixth site, the Olympia waterfront, was not a primary study site but was included as a target area for some of the study objectives.

5.1.2 Colony history

The history of all sites is reviewed in Speich and Wahl (1985). Data for 1983 were obtained from S.M. Speich (unpubl. obs.). Early information for Tacoma was obtained from C. Sheridan (pers. comm.). S.P. Thompson provided data from 1983 and 1984 for Smith Island. J. Galusha (pers. comm.) provided data for Protection Island.

5.1.3 Colony census

The approximate total number of nesting Glaucous-winged Gulls in each study colony was determined by counting nests or, where nests were not accessible for counts, by estimating numbers of breeding adults.

Surveys of the nest contents were made at each study site. In some cases the survey included all nests present, however, in most cases the survey was a sample of the nests in the colony. When the census was a sample, the nests were chosen at random from part of the colony. During each census nests were observed to determine their contents, which were recorded as: empty (stage of nest completion noted); number of eggs; number of nestlings; young out of the nest; and the number of dead eggs and dead young present. Notes of pipping eggs were recorded. Notes were taken on the condition, stage of development, and any determinable cause of death for all dead eggs and dead nestlings found.

Nest contents surveys were made on the following days at each colony. The number of nests censused on each date are in parentheses. There were five surveys of Smith Island: 16 May (n=103 nests), 14 June (153), 23 June (62), 4 July (104), and 11 July (121). There were four surveys of nearby Minor Island: 16 May (103), 5 June (168), 13 June (112), and 23 June (162). The nest contents surveys of Smith Island and Minor Island were only samples of the colonies there. There were seven censuses of the Seattle, Smith Cove colony. Each census was of all nests, and all were marked, on one or more roof tops. Not every roof was censused on each visit. The visits were on: 4 May (5, there were no other nests present), 22 May (36), 1 June (106), 17 June (150), 21 June (120), 2 July (140), and 19 June (69). The Tacoma roof top colony was censused eleven times, each census included all known nests and nest sites on the roof: 7 May (120), 15 May (159), 16 May (154), 22 May (167), 25 May (180), 1 June (173), 5 June (193), 10 June (197), 21 June (162), 29 June (150), and 10 July (88). The only part of the Olympia breeding population censused were those birds nesting on a warehouse roof. On each of the six censuses, all nests on the warehouse roof were observed: 2 June (17), 11 June (32), 17 June (31), 24 June (31), 1 July (32), and 13 July (31). The Shelton colony was sampled seven times: 8 May (123), 11 May (61), 16 May (144), 26 May (109), 2 June (129), 18 June (132), and 3 July (71). And the Goose Island colony was sampled three times: 18 May (702), 24 May (188), and 6 June (728).

5.1.4 Marking nests

Two methods were used to mark gull nests. On Smith Island, at Shelton, and on Goose Island the nests were marked by placing 50 or 25 cm numbered stakes next to each sample nest. Nests on the dock of the Tacoma colony were marked by placing numbered plastic tags near each sample nest. Nests on the roof-top colonies in Seattle, Tacoma, and Olympia were marked by writing the nest number on the roof, ventilators, or sky lights. At the first three colonies, a sample of all the nests present were marked, while at Seattle, Tacoma, and Olympia all the nests on the roof tops were marked.

5.1.5 Marking young

Nestlings and walking chicks were marked at each site. When small, young birds were marked with colored pens on their ventral side or on their foot webs, or small pieces of colored plastic tape were placed around their tarsometatarsus, for later specific identification. When the young were bigger they were banded with standard serially-numbered aluminum U.S. Fish and Wildlife Service bird bands. The marking and banding of young birds established individual identity of birds when measurements were taken, and were used to determine the survivability of young associated with particular nests.

5.1.6 Censuses of young

Young were counted during nest censuses of study plots. Initially young were in or near their nests and censuses were made by nest. When most or all young were out of their nests, counts of young in portions of the colony were made and the average number of young per nest was calculated. Later in the season when young were flying, counts were made of the entire colony area at some sites.

5.1.7 Collection of specimens and necropsy

Glaucous-winged Gulls were collected from the six study sites (Figure 13). Adults, whole eggs and egg fragments were collected. Adult females and their clutches of three eggs were collected (n=43) from each of five study sites: Goose Island (10), Shelton (11), Tacoma (9), Seattle (5), and Smith Island (8). Females and their three-egg clutches were chosen to provide a data set consistent between sites. Twenty-five birds were collected from target areas (Seattle, Tacoma, and Shelton) and 18 from reference areas (Goose and Smith Islands). Additional adults were collected when they were found dead in the colony or showed abnormal behavior: Goose Island, 2 males found dead and one found weak; Shelton, one female found dead and one male found dead; Olympia, one male found injured and weak; Tacoma, one male and three eggs collected from its nest; Seattle, one female observed dying (convulsions) at nest; and Smith Island, one male collected without its eggs, one male and one female found weak, and a tumor was removed from a male trapped from its nest (banded and released).

All collected eggs were later blown and the contents frozen. All eggshell fragments are now in the Western Foundation of Vertebrate Zoology (WFVZ), Los Angeles, and whole eggshells are in the WFVZ and the Burke Memorial Washington State Museum, University of Washington, Seattle.

Birds were captured alive in nest traps and carried to the necropsy area. Blood was taken from the brachial vein in hematocrit tubes with a syringe needle for later examination. Air dried blood smears were prepared from blood collected in one of the hematocrit tubes. Birds were killed with an inhalation overdose of ethyl ether followed by opening of the body cavity and exanguination by opening the heart. At most sites, birds were killed individually just before necropsy. Necropsies were initially performed by a pathologist (D.M. Fry) and then by personnel who had been trained and assisted in the initial necropsies by Dr. Fry. Each organ was located and described, including weights of whole carcass and liver, and dimensions of most organs. The entire viscera were removed, lumens of hollow organs opened, and viscera were preserved in 10% neutral formalin. The proximal third of the femur was removed and included for a sample of bone marrow and indication of extent of medullary bone. Salt glands were removed from the top of the skull, and the brain was removed intact and fixed in formalin. Tissues to be saved for future chemical analysis were removed during necropsy and saved in solvent rinsed glass bottles with foil lined caps. Necropsy descriptions were completed for each bird at the time of gross dissection.

Tissues saved for histopathology included the entire alimentary canal, respiratory tract and lungs, heart and major vessels, urogenital system, a sample of pectoral muscle, proximal femur, uropygial and nasal glands, adrenals, thyroids, and brain. Any additional tissues noted as unusual at gross examination were included for histology.

5.1.8 Histopathology

Fixed tissues were embedded, sectioned, and stained by the technical staff of the Dept. of Veterinary Pathology, University of California at Davis, and examined by Dr. Lowenstine. A complete description of the histopathology of each bird was prepared at the time of microscopic examination.

5.1.9 Hematology

Blood samples were refrigerated after collection and sent to the Veterinary Reference Laboratory, Salt Lake City, Utah for analysis. A complete Avian Blood Panel (a broad screening of blood parameters including: white blood cell count, red blood count, hematocrit and differential, parasite quantification, serum glutamin oxalacetin transaminase level, total protein, lactic dehydrogenase level, creatinine level, calcium level, glucose level, and uric acid level) was conducted for each bird. The results of the panel were compared with the gross and histopathological findings for each bird, and average values were calculated for birds from each location.

We collected blood samples from the adults for which clutches were also collected. The number from each site was: Goose Island (7), Shelton (7), Olympia (1), Tacoma (6), Seattle (5), and Smith Island (7).

5.1.10 Reproductive success measurements

Data for five measurements of reproductive success were obtained: clutch size, hatching success, fledging success, nestling growth rates and

breeding chronology. These were determined for the six study sites (Figure 13). Clutch size was determined when nests were inspected, as was the number of eggs that hatched. Growth rates were calculated by weighing nestlings on two or more visits to nests. Fledging success was inferred from the presence of large nestlings in proximity of nests. Values from each colony were compared between Puget Sound colonies and similar measurements reported by other researchers.

5.1.11 Measurements of eggs, young, and adults

Several different measurements were made of adults, nestlings, and eggshells. These measurements were made in the same manner at each site.

All whole eggs and eggshell fragments (more than half an eggshell) collected and salvaged were measured for thickness. Whole eggs were measured at the egg's equator, and fragments as near the equator as possible. All thickness measurements, shell and membrane, were made to the nearest 0.001 mm. All eggshell thickness measurements were made by personnel of the Western Foundation of Vertebrate Zoology, using a modified Bench Comparator with a Federal Dial Indicator.

Nestlings were weighed twice to obtain data to determine mean growth rates for young from each colony. Nestlings with weights in the straight line portion of the generalized growth curve were used. The lower and upper weight limits of the straight line growth curve were derived from the daily mean weights of nestlings in Vermeer (1963). From these data, weight values in the range of 150 to 850 grams were generally used to calculate growth rates.

Captured adults were measured to determine their sex, as we attempted to collect only females and their clutches. It was usually possible to determine the sex of captured birds by weighing each bird. Birds weighing less than one kilogram were classified as females, and those weighing more than one kilogram as males (W. Reed, pers. comm.).

5.1.12 Observations for abnormalities

All eggs and nestlings handled were examined for abnormalities. The surface condition and shape of all eggs were observed. All the soft parts and plumage of all nestlings handled were examined. The soft parts and plumage of adult males that were trapped and released were examined. The external surface (feathers, soft parts, and body surface) of adult females collected were carefully examined. The numbers of adults, young, and eggs examined for abnormalities are listed in Table 13.

5.1.13 Observations of mortality

Observations of dead eggs, eggshell fragments, dead nestlings and young, and dead adults were recorded. Notes on the location and condition of dead specimens were recorded. The specimens were examined to determine their stage of development, in the cases of eggs and young, and the cause of death, if possible. Particular attention was given to looking for "unusual" mortality of adults, young, and eggs.

Table 13. Numbers of Glaucous-winged Gull adults, young and eggs examined for abnormalities, western Washington, 1984.

Location	Adults necropsied	Young banded	Numbers observed		
			Adults(1)	Young(2)	Eggs(3)
Smith Island	9	115	<100	<160	<300
Seattle	6	52	<100	<200	<350
Tacoma	10	139	<400	<400	<650
Olympia	1	54	<50	<70	<100
Shelton	12	41	<150	<150	<350
Goose Island	12	172	<150	<200	<1,500
Totals	50	573	<1,100	<1,180	<3,250

1. Observed with binoculars and telescope in colony. Based on maximum number present in colony.
2. Observed in or near nests during nest contents surveys. Based on maximum number present in colony.
3. Observed in nests during nest contents surveys. Based on maximum number present in colony.

5.2 Results and Discussion

5.2.1 Population status and changes

Each Glaucous-winged Gull colony studied was censused one or more times, by ourselves or other investigators. For most colonies there are only limited historical data pertaining to numbers nesting (Speich and Wahl, 1985). All historical data reported below are summarized from Speich and Wahl (1985) unless otherwise noted.

Smith Island. This colony site was first visited in 1792 (first bird records). Although Black Oystercatcher (Haematopus bachmani) were observed, captured, and eaten, no mention of gulls nesting was made in these early accounts. The first count of birds is from June 1963, when 300 gulls were recorded. Other counts were in 1967 - 500 birds, 1979 - 220 birds, and 1982 - 1,060 birds. A count of adults on 11 July 1984 revealed 1,125 birds on the island (S.P. Thompson, pers. comm.). The data suggest the population nesting on Smith Island has increased by a factor of perhaps four since 1963.

Seattle. It is difficult to census the Seattle "colony". It consists of several nesting locations scattered from the Duwamish Head, the port waterways, along the Alaskan Way waterfront, in downtown Seattle, and Smith Cove (Piers 90 and 91). The gulls nest on the roofs of warehouses, on pilings and old docks, and on the buildings in the financial district of Seattle. Notes on the history of the birds in the downtown area of Seattle were reviewed by Eddy (1982). Eddy first observed birds in the downtown area of Seattle in 1946, recording about 40 nesting birds. In 1966 the numbers had grown to an estimated 70 birds nesting in the area, and over 100 birds in 1971. Over 100 birds were also observed in the area in 1982. Nearly 230 birds nested on Pier 28 in 1981, and also in 1982, but part of the nesting area at the pier was removed prior to the summer of 1984 and most birds left that site. In 1982 over 120 nesting birds were found along the waterfront from Pier 36 to Pier 71. The gulls nesting in Smith Cove, Piers 90 and 91, were first censused in 1977, with about 160 birds nesting. In 1981 there were about 280 birds nesting in the Smith Cove area. In June 1984, we found over 300 birds nesting on buildings in the Smith Cove area. Thus, there is evidence the population in the Seattle area has increased, but precise quantification is difficult. The population in the Seattle port area may now number about 1,000 breeding birds.

Tacoma. The Glaucous-winged Gull was first reported as a possible breeding bird in Commencement Bay in 1928, when a bird was collected. The first definite record is of an egg collected 28 July 1945 at the mouth of the Puyallup River by J.W. Slipp (C. Sheridan, pers. comm.). He reports the species was not nesting in the area prior to 1945. Alcorn (1949) reported 20 nesting birds on the waterfront in 1948. An incomplete census of the waterfront by boat in June 1982 revealed over 550 nesting birds (Wahl and Speich, 1984). In June 1984, in a more complete census than that of 1982, we recorded over 1,230 nesting birds on the Commencement Bay waterfront. The numbers of this species nesting on the Commencement Bay waterfront have certainly increased.

Olympia. The first known data on numbers nesting are from 1976, when 18 birds were observed. Other reports are, 1978 - 30 to 40 birds, 1979 -

20 to 24 birds, 1981 - 35 birds, and 1982 - 30 birds. Censuses in June 1984 of the area revealed about 160 nesting birds. This population has increased, but the rate and period are hard to define.

Shelton. There are few data available from this site. In 1982, 48 birds were present. Our censuses in June 1984 found 152 birds nesting. From this small amount of data no conclusions can be made of the history of this colony.

Goose Island. It is difficult to interpret the data available from this site. Glaucous-winged Gulls were present and nesting from 1954 through 1958, but the numbers are unknown. Birds were nesting there in 1972. In 1975 an estimate of 7,000 birds was made on one count, and 2,000 and 1,300 on others. Counts from 1977 were 7,000 and 4,000 birds. The birds were present and breeding in 1979, 1980, and 1982. Close to 780 nests were counted on part of the island in 1984. An estimated 100 nests were on the remaining part of the island where nests were not counted, indicating close to 1,800 birds were nesting on Goose Island. The data from Goose Island are not adequate for determining trends in the numbers of birds nesting there.

Summary. It is clear that at least the colonies at Smith Island, Tacoma, and Olympia have increased. Simultaneously, records show that the number of gulls nesting on Protection Island has increased. In 1938 there were less than 200 birds nesting on the island (R. Newcomb, pers. comm.). Now there are over 14,000 nesting gulls there (J. Galusha, pers. comm.). Limited data from other colonies in the inland marine areas of Washington indicate that increases of gulls nesting at other sites has occurred (Speich and Wahl, 1985). We found no evidence that the numbers nesting at any of these colonies have decreased.

5.2.2 Breeding chronology

There was a difference in the time that egg-laying began among the colonies (Figure 14). Overall there was a span of about thirty days between the start of egg-laying of the earliest and latest colonies. Goose Island in Grays Harbor appeared to be the first colony to begin nesting, in the last part of April, based on the high proportion of nests with eggs found on our first visit in mid-May. The Goose Island colony was about ten days ahead of the next colony to nest, Tacoma (about 5 May). Egg-laying in the Shelton colony apparently started a few days after the Tacoma colony. These colonies were followed by a grouping of the remaining colonies, which all started to nest after the middle of May. Of this group - Olympia, Seattle, Minor Island, and Smith Island - it appears that the gulls at Smith Island colony generally laid eggs the latest.

In addition to between-colony differences, subtle differences in the timing of egg-laying within colonies were found. At Tacoma it was clear the birds nesting on the abandoned dock started nesting a few days before the main roof top colony. The colony nesting on Minor Island, about a half kilometer from Smith Island, was a few days ahead of the colony on Smith Island in the start of egg-laying.

We cannot offer an explanation for the observed differences in the start of egg-laying. Birds at one reference site, Goose Island, were the

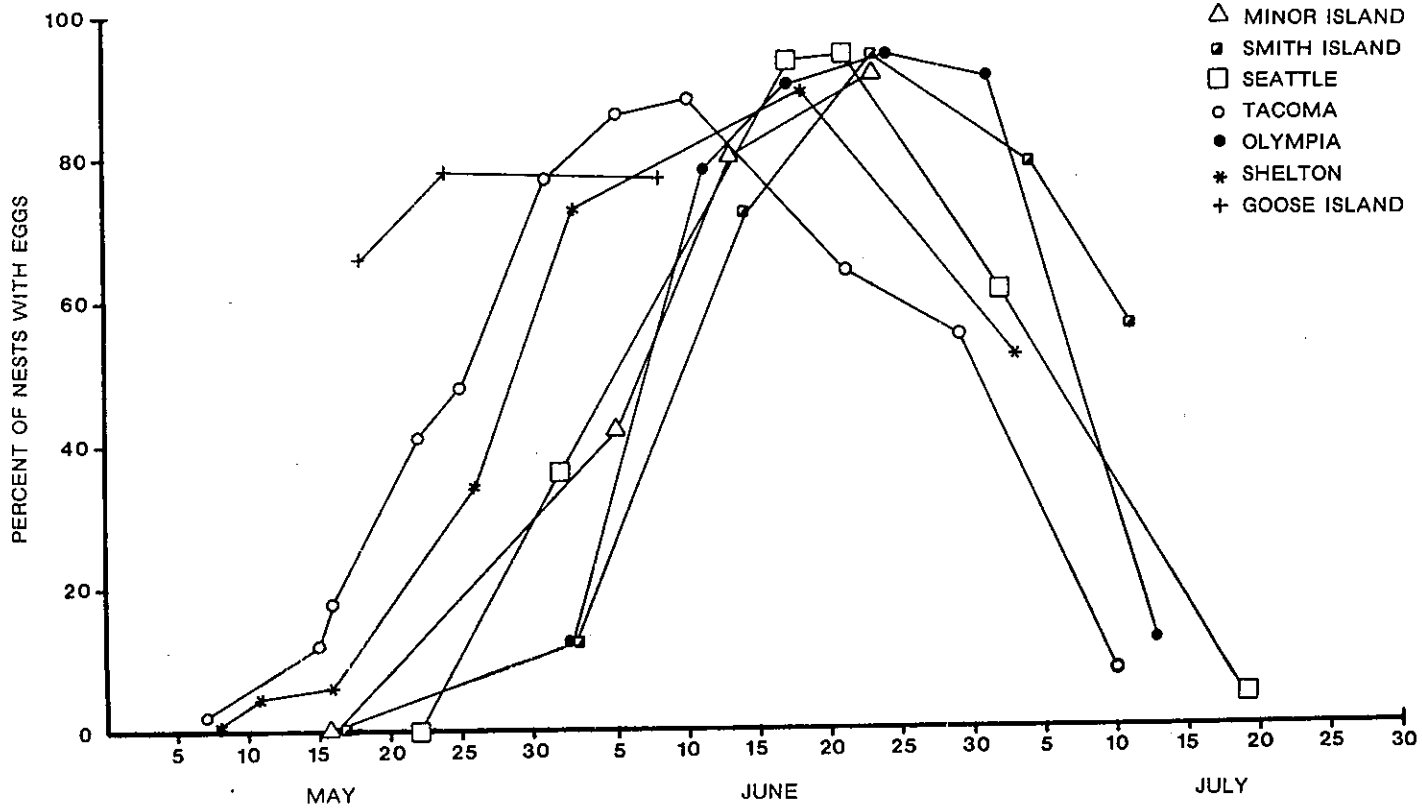

Figure 14. Percent of nests with eggs at Glaucous-winged Gull colonies in western Washington, 1984.

first to start nesting, and birds at the other reference site, Smith Island, were among the last to nest. Birds at target sites were intermediate to those at reference sites in their start of egg-laying.

5.2.3 Reproductive success

Clutch size. The mean clutch size at nests where at least one egg was laid varied from 2.21 to 2.90 eggs per clutch (Table 14). There was significant variation among the mean values from the colonies (ANOVA, $p<.05$) and in clutch size distribution among the sites (chi-square, $p<.05$); clutch size was generally higher at target sites compared to reference areas. Clutches of three eggs were most common, followed by two, one and four.

The clutch size frequencies observed in this study (Table 14) were similar to those obtained by Vermeer (1963) during a study of Glaucous-winged Gulls nesting on Mandarte Island, British Columbia in 1962. Other similar data were obtained from the same colony earlier, 1959-1961 (Drent et al., 1964). Although the distributions observed in these two studies were similar there was significant heterogeneity in the clutch size distributions between the years (chi-square, $p<.05$). There were no clutches of four observed during the studies on Mandarte Island (Vermeer, 1963).

Clutch size and clutch size distribution were determined for Western Gulls (Larus occidentalis) nesting on Southeast Farallon Island off San Francisco, California during four years, 1968, and 1970-1972 (Coulter, 1973). Again, although the clutch size distributions were similar there was significant heterogeneity between the sample years (chi-square, $p<.05$).

Differences in the timing of our visits to sites may have been responsible for intersite differences since the clutch size of a colony is dependent upon the frequency of the different clutch sizes present in the colony at the time of survey. Thus, a survey of nest contents early in the egg-laying cycle of the colony will result in a clutch size distribution skewed to the smaller clutches and a smaller calculated mean clutch size than if the survey were later in the egg-laying cycle. At the other end of the egg-laying cycle, after most of the clutches are completed, eggs are lost from nests, resulting in lower calculated clutch size values. In colonies where nest contents were often observed we were more likely to observe all the eggs in the clutch before one or more were lost, resulting in apparent larger clutches. The amount of egg loss is also dependent upon the structure of the nesting substrate. On Goose Island many nests were lost to moving sand and high tides. On roof top colonies the nests and eggs were nearly always in open exposed positions, making them more vulnerable to egg loss during periods of disturbance and the resulting territorial disputes between displaced adults.

Data collected on Mandarte Island and the Farallon Islands show significant variation between years in clutch size distribution and mean clutch sizes. As this type of variation is documented between years at individual colonies, it is not surprising to find similar variation between colonies within any one year. Additionally, the studies on Mandarte Island and the Farallon Islands were more complete and the clutch size distributions and mean clutch sizes were likely accurate. However, despite the significant variation between our study sites, the clutch size

Table 14. Clutch size distribution and mean clutch size observed for nests where at least one egg was laid at Glaucous-winged Gull colonies, western Washington, 1984.

Location		Clutch size 1	2	3	4	Total clutches sampled	Mean clutch size(s.d.)	Survey date(s)
Smith Island	n=	14	37	59	0	110	2.41	14 June
	%=	13	34	54	0		(.708)	
Seattle	n=	3	22	95	2	122	2.79	June/July
	%=	2	18	78	2		(.502)	
Tacoma	n=	4	21	175	0	200	2.86	June/July
	%=	2	11	88	0		(.406)	
Olympia	n=	0	3	27	0	30	2.90	May/June
	%=	0	10	90	0		(.305)	
Shelton	n=	8	29	83	0	120	2.63	18 June
	%=	7	24	69	0		(.609)	
Goose Island	n=	133	219	259	1	612	2.21	8 June
	%=	22	36	42	<1		(.777)	

distributions, and mean clutch sizes all indicate that the colonies we observed in western Washington were reproducing in a "normal" manner. That is, there were no obvious and obtrusive reproductive problems observed in the egg-laying of the colonies.

Supernormal clutches. In this study we observed three clutches with four eggs each (Table 14). These are referred to as supernormal clutches and have been observed in several gull and tern species (see Conover, 1984; and Conover and Hunt, 1984 for recent reviews and discussion). The normal clutch size for gulls is one to three eggs. In other species of gulls, clutches with more than three eggs were in nests with female-female pairs or less commonly polygynous matings. Where this occurred, there was a shortage of males in the breeding colonies. The experimental removal of males from a gull colony resulted in an increase in female-female pairings (Conover and Hunt, 1984). However, it is not known why there is a lack of males, or an excess of females, in gull populations. It has been suggested that DDT can feminize male gull embryos, so that the resultant "female" birds do not breed, and the population sex ratio becomes changed (Fry and Toone, 1981).

The occurrence of two supernormal clutches in Seattle is interesting in light of data collected earlier at the Seattle gull colony. In 1979 (Table 15), G. Eddy (pers. comm.) made a survey of the nest contents of the Glaucous-winged Gulls nesting on warehouse roofs in Smith Cove. He found that 14 (21%) of the 67 nests surveyed held clutches of four eggs. He repeated the survey in 1981, observing 147 nests, but none held four eggs. Considering the extent of occurrence of supernormal clutches in other gulls and the documented occurrence of female-female pairs in these species and at nests with supernormal clutches, female-female pairs are the most likely source of the supernormal clutches observed in Seattle.

Hatching success. One measure of reproductive success is hatching success, the percentage of eggs laid that hatch. All observations to determine hatching success were of marked nests, selected for having complete or near complete clutches of three eggs. This part of the study was not designed to compare the hatching rates between clutches of different sizes. However, the samples from Seattle, Tacoma, and Olympia represent all nests that could be followed accurately. A very low incidence of hatching, associated with high levels of PCB and DDE residues in eggs, was one of the primary factors identified in the reproductive failure of Herring Gull populations from the Great Lakes (Gilbertson, 1974).

In this study (Table 16) the hatching rate varied from 52% at Smith Island to 79% at Tacoma. At Smith Island and Shelton the longer interval between different nest contents determinations could have biased the observed hatching rate downward. Unfortunately few trips were made to Goose Island and the loss of many marked nests under drifting sand did not allow a determination of hatching rate there. However, casual observations strongly suggest that it was comparable to that observed at the other sites. There was not a consistent difference in hatching rates between target and reference areas.

Two studies of gull hatching rates, each conducted over four years, report values consistent with this study. Glaucous-winged Gulls on

Table 15. Clutch size distribution in the Seattle (Smith Cove) Glaucous-winged Gull colony.

Total clutches observed	Year		Clutch size 1	2	3	4	Source
67	1979	n=	8	15	30	14	G. Eddy pers. comm.
		%=	12	22	45	21	
147	1981	n=	15	48	84	0	G. Eddy pers. comm.
		%=	10	33	57	0	
122	1984	n=	3	22	95	2	This study
		%=	2	18	78	2	

Table 16. Hatching and fledging rates observed at Glaucous-winged Gull colonies, western Washington, 1984. Data gathered from gulls at marked nests only.

Location	Number of nests	Number of eggs	Eggs hatched/ nest	Percent eggs hatched	Maximum number fledged/ nest	Maximum number fledged/ eggs laid
Smith Island	31	91	1.52	52	----(1)	----(1)
Seattle	82	225	1.99	72	1.28(2)	0.49(2)
Tacoma	153	440	2.26	79	1.53(3)	0.53(3)
Olympia	30	87	1.77	61	1.43	0.49
Shelton	20	59	2.15	75	----(1)	----(1)

1. Insufficient data
2. Based on sub-sample of 53 nests and 139 eggs
3. Based on sub-sample of 109 nests and 314 eggs

Mandarte Island in British Columbia had hatching rates from 57-71% (Drent et al., 1964). Hatching rates of Western Gulls on the Farallon Islands ranged from 69-79% (Coulter, 1973).

Fledging success. Fledging success was determined for three colonies (Seattle, Tacoma, and Olympia), where every nest in the portions of the colonies studied was marked and observed repeatedly (Table 16). But, the rates determined were the maximum possible rates because some mortality may have occurred after observation but before fledging. Actual rates may be somewhat lower. It was not always possible to follow nestlings to near fledging, as they wander from their nests. The rate that young fledged per egg hatched was: Seattle - 49%; Tacoma - 53%; and Olympia - 49%. The average number of young fledged from each nest was, respectively, 1.28, 1.53, and 1.43.

Drent et al. (1964) reported fledging rates of Glaucous-winged Gulls on Mandarte Island as ranging from from 31% to 61%. Numbers of young fledged per nest ranged from 0.5 to one over a three year period. The rates we observed were similar or higher. Low fledging success rates, linked to high residues of PCBs and DDT in eggs, were found in Great Lakes Herring Gull populations that experienced low reproductive success in the early 1970s (Gilbertson and Hale, 1974b; Teeple, 1977).

Growth rates. The mean growth rate of young was determined at each of the six study sites (Table 17). There was considerable and significant variation between the sites (ANOVA, $p<.05$). The values from Goose Island and Shelton were the smallest.

There are probable explanations for the observed low growth rates of young from Goose Island and Shelton. The Goose Island weighings were made when the nestlings were older than at other sites and this difference in age was likely to have biased the growth rate measurement downward. Young birds from Shelton, however, were growing slower than young from other sites. Indeed, three young were loosing weight (-7.0, -29.2, and -25.0 g/day). Weight loss was not observed at any other site. The most likely explanation is that adults at Shelton were having difficulties obtaining food for their young late in the nesting season. Our sample size is insufficient to allow comparisons of growth rates of young by brood-size, although such differences undoubtedly exist for the species we studied.

The growth rates were intended to be a general indicator of the health of the birds at the colonies, or more precisely, a measure of the ability of adults to find sufficient food for their young. It is reasonable to expect adults to vary in their ability to provide food for their young. In studies of the Glaucous-winged Gull (Vermeer, 1963) and Western Gull (Coulter, 1973), young from larger broods grew more slowly than young from smaller broods.

5.2.4 Eggshell thickness

Eggshell thinning in birds has been well documented and related primarily to residues of DDE in the eggs (Cooke, 1973). Peakall (1975) reported that gulls are only moderately sensitive to DDE-induced eggshell thinning. However, a number of studies have reported significant eggshell thinning in various gull species that was correlated to levels of DDE

Table 17. Growth rates of young Glaucous-winged Gulls from six study sites, western Washington, 1984.

Location	Number young measured	Mean growth rate grams/day (s.d.)	Range
Smith Island	12	33.3 (6.36)	22.1 - 43.1
Seattle	19	31.8 (10.77)	10.0 - 47.9
Tacoma	43	33.4 (7.31)	9.0 - 47.0
Olympia	10	29.3 (8.26)	15.1 - 41.2
Shelton	12	9.6 (20.54)	-29.2 - 41.0
Goose Island	12	20.7 (8.92)	9.0 - 29.3

(Anderson, 1970; Gilbertson, 1974; Gilman et al., 1977; Hickey and Anderson, 1968; Faber and Hickey, 1973; Cooke, 1979).

Glaucous-winged Gull eggs were collected from five study colonies from western Washington in 1984 (Table 18). In total 111 egg specimens were collected, salvaged, and measured. There was significant variation in mean thickness of whole eggs among the five study sites (ANOVA, $p<.05$).

Eggs from the two reference sites, Smith Island and Goose Island, generally had thicker eggshells than those at the three target sites, Seattle, Tacoma, and Shelton. When data from the reference areas were combined, the mean thickness was significantly greater than that of the target areas (t-test, $p <.001$).

It has been recognized that coastal Washington is the primary area of contact and hybridization between the Western Gull and the Glaucous-winged Gull (Hoffman et al., 1978). Therefore, our samples were of several different morphs and it was not clear to which species our eggshell thickness measurements should be compared, the Glaucous-winged Gull or Western Gull. The hybrid zone is also evident in Puget Sound, though birds in Puget Sound and especially in the San Juan Islands are clearly more similar in coloration to the Glaucous-winged Gull morph than the Western Gull. The Goose Island study site is clearly in the primary hybrid zone, while the Puget Sound and Smith Island sites are in the less evident hybrid zone area. Many of the birds we collected were "intermediate" morphs and a few were closer in coloration to the Western Gulls than Glaucous-winged Gulls.

Fifty-two Glaucous-winged Gull eggs (one egg from each clutch was measured) collected in the San Juan Islands prior to 1947 (between 1898 and 1937) were measured to provide reference values to determine degree of eggshell thinning (Table 18). Only values from the 1984 Seattle, Tacoma, and Shelton samples were significantly thinner than the pre-1947 Puget Sound measurements (Table 18; t-test, $p<.001$). The pre-1947 San Juan Island thickness value we used was significantly thinner than the pre-1947 Pacific Northwest thickness value reported by Henny et al. (1982) apparently as a result of Henny's measurements being more representative of Western Gulls.

5.2.5 External Abnormalities

Four different types of abnormalities were observed in this study. These involved egg color, pigmentation of young, deformities, and tumors (see Table 13 for number of adults, young, and eggs examined).

Egg color. One egg was found that was uniformly colored greenish-blue. This egg was one of three eggs in a nest on Goose Island. The other two eggs were of normal color and color pattern.

Pigmentation of young. Of the many young birds observed a few were found with irregularly shaped areas lacking pigment on the feet, toes, toe webs, and lower leg (below ankle). These areas were off-white pink-colored, and contrasted sharply with the normal dark color of the legs and feet. The splotches covered about a third of the pads. No other abnormalities were associated with the splotches. Out of the 52-200 young

Table 18. Eggshell thickness (with membrane) of whole eggs of Glaucous-winged Gulls, western Washington, 1984.

Location	Number eggs measured	Mean thickness mm (s.d.)	Percent change from pre-1947*	Statistical significance
Smith Island	16	0.384 (.035)	-3	NS
Seattle	13	0.354 (.032)	-10	<.001
Tacoma	20	0.360 (.028)	-9	<.001
Shelton	29	0.362 (.025)	-8	<.001
Goose Island	33	0.388 (.022)	-2	NS
All sites (above)	111	0.372 (.029)	-6	<.001
Pre-1947, San Juan Islands	52	0.395 (.025)	---	-

* -Measurements from San Juan Islands.

birds examined from Seattle (Table 13), one bird had abnormal pigmentation and only two birds out of 139-400 young birds at Tacoma had this condition. Pigment abnormalities were not seen at other sites.

Deformities. There was only one external structural deformity observed in all the young birds examined. In the Seattle colony one young bird had deformed toes. The outer toes on each foot had the last segment permanently bent inward at a forty five degree angle at the last toe joint. There was no other associated abnormality.

Tumors. One adult male captured from its nest on Smith Island had a small growth at the base of the lower mandible. The growth was about one centimeter in diameter. It was removed and preserved for analysis. Blood samples were also taken and the bird was released. The bird appeared healthy otherwise.

5.2.6 Hematology

A complete avian hematology profile was performed on each of the birds collected for histopathology (Table 19). No values for "normal" serum constituents of gulls exist in the literature, and this study has presented a good opportunity for screening what outwardly appeared to be healthy birds. The variability within each group of tests was marked, much greater than was expected for normal birds. Averages of scores from sites were not significantly different from each other as variations between individuals were high. Correlations of blood chemistry and pathology could be made in specific individual cases. The birds with severe yolk peritonitis and a bird with the bone perforating the gizzard, for example, had high white blood cell counts, but serum parameters of other birds were variable and less predictive of general health than histology.

The hematocrit, or packed cell volume, is the percent of total blood cells per ml of blood and is a good measure of anemia. Seabirds generally have hematocrits in the range of 50-55%, while most land birds have slightly lower values. The reported values of 48.7 to 54.8 (Table 19) fall within the expected range. Individual birds with low hematocrits could be anemic, or could have had tissue fluids mixed with blood during vein puncture. Only one bird had a hematocrit lower than 45, the bird from Smith Island with the chicken bone penetrating the gizzard, body wall and thigh. This bird was a candidate for significant loss of blood, and the finding of 42%, even then, was not extremely unusual.

Values for the red blood count represent the number of red cells per cubic millimeter of blood and generally reflect the hematocrit. In cases of blood disease, the values may differ if unusually small cells are present due to regenerative processes or disease.

White blood counts are determined in birds by staining a suspension of blood cells with an eosin containing stain and counting heterophils. An estimate of the number of white cells is made and a corrected count is calculated from the proportions of white cell types determined from a differential count made from a blood smear. Only the corrected white cell count is given in Table 19. The populations of white cells can, in a general way, help predict the course of disease and the type of infection present. Monocytes generally increase in cases of chronic inflammation,

Table 19. Mean hematology values of adult Glaucous-winged Gulls collected at sites in western Washington in 1984. Values represent results of an avian total function profile performed by Veterinary Reference Laboratory, Salt Lake City, Utah.

Parameter	Locations				
	Goose Is.	Tacoma	Shelton	Seattle	Smith Is.
No. examined	7	6	7	6	6
Blood cell indices:					
Hematocrit (%)	52.3	50.7	53.4	54.8	48.7
Red bl. count	2.8	3.2	3.4	3.3	4.2
Corr. white blood count (thou/mm3)	10.2	11.7	9.7	8.8	11.1
Heterophil count (#/mm3)	6529	8230	4817	5538	8681
Heterophils (%)	58	67	50	69	74
Lymphocytes (%)	35	27	49	26	20
Monocytes (%)	4	3	4	4	5
Eosinophils (%)	1	1	2	1	1
Basophils (%)	5	3	2	1	2
Serum glutamin oxalacetin transaminase level (Int. Un./L)	448	209	285	280	458
Total protein (gm/dl)	4.5	4.2	6.1	5.1	3.7
Creatinine (mg/dl)	.25	.28	.27	.16	.11
Calcium (mg/dl)	14.3	12.4	16.0	12.8	13.8
Uric acid (mg/dl)	7.9	6.6	11.6	12.9	13.4

heterophils are generally elevated in cases of acute inflammation, and lymphocytes increase with sub-acute infections. The proportions of basophils and eosinophils reported in avian blood may be very skewed from predicted values as there is great species variation in staining properties and cell morphology. All of the average values given in the table are within the "normal" values given by Veterinary Reference Laboratory and the excellent hematology chapter by Leonard (1982).

Total protein values are very predictive of health of an individual bird with increases occurring during periods of dehydration, and decreases occurring with infectious or metabolic diseases. The values given in Table 19 are within expected values with the exception of birds from Shelton. Birds which are incubating may subject themselves to dehydration and starvation and some of the values from individual birds may reflect this. High total protein may be physiologic during egg formation with the mobilization of albumin and yolk proteins, but these birds had all completed their clutches.

Uric acid levels are reflective of nitrogen metabolism and recent carnivorous meals. Uric acid is the major nitrogen excretory product of birds, and elevated levels usually indicate a recent large intake of protein, but may indicate kidney malfunction. Low levels of uric acid indicate liver disease or a low protein diet. Only one bird had a very remarkable uric acid level, the bird from Smith with the bone through its gizzard, had a anomalously high uric acid level of 39.5 mg/dl.

Calcium levels are generally very closely regulated by the parathyroid glands and fall in the range of 8-14 mg/dl (Table 19). Many of the birds in this study had high calcium levels, undoubtedly reflective of normal physiology during eggshell formation and mobilization of medullary bone.

In summary, the hematology values of the population as a whole were reasonably uniform and appear to represent normal values.

5.2.7 Necropsy and histopathology

Most of the birds necropsied in this study were adult breeding females, a sample which represents the healthiest birds in the population. Injuries, disease, or starvation would all contribute to lowered breeding success. By selecting primarily birds which were incubating three egg clutches, we automatically selected for birds with minimal effects of stress, birds which could compete successfully for mates and breeding sites, and birds which were in sufficiently good condition to lay complete clutches of eggs. It is particularly noteworthy, therefore, to find significant differences between birds from different breeding sites, and these differences probably reflect significant environmental differences or food habit differences between areas of Washington. By selecting a narrow range of birds, and collecting at the same period of the breeding cycle, much of the possible confounding variability inherent in "random" sampling has been avoided.

A summary of gross pathological conditions observed during necropsy are summarized in Table 20. Histological results are summarized in Table 21.

Table 20. Number and percent of adult Glaucous-winged Gulls with specified gross lesions observed at necropsy.

Condition	Locations									
	Goose Is.		Tacoma		Shelton		Seattle		Smith Is.	
	No.	%	No.	%	No.	%	No.	%	No.	%
No. examined	7	100	6	100	10	100	6	100	7	100
Tapeworm	4	58	5	83	8	80	3	50	3	43
Nematodes:										
Small	2	29	2	33	5	50	4	67	5	71
Large	1	14	0	0	3	30	3	50	3	43
Tetrameres	3	43	2	33	3	30	0	0	3	43
Crop paralysis	0	0	1	17	0	0	1	17	1	14
Gizzard:										
Erosions	3	43	3	50	6	60	1	17	3	43
Hemorrhages	1	14	3	50	2	20	1	17	3	43
Enlarged liver	1	14	0	0	6	60	6	100	3	43
Kidney foci	1	14	1	17	1	10	0	0	0	0
Traumatic lesion (type or location)	0	0	3 1	50(feet) 17(pox)	4 1	40(feet) 10(bone)	0	0	0	0
Bact. infects. (location)	0	0	1	17(yolk)	1	10(bone)	1	17(yolk)	0	0
Spleen enlargm.	1	14	2	33	1	10	1	17	2	29
Right oviduct (RO):										
No. examined	6		6		7		6		6	
RO present	1	17	6	100	6	86	3	50	5	83
RO > 10 mm	1	17	6	100	2	29	1	17	3	50

Table 21. Histopathological conditions in sampled adult Glaucous-winged Gulls.

Condition	Locations				
	Goose Is.	Tacoma	Shelton	Seattle	Smith Is.
No. examined	7	6	7	6	6
Liver:					
Hepatitis	5	4	2	2	4
Fatty changes	3	6	4	0	2
Amyloidosis	1	0	2	1	2
Hepatocellular iron	6	5	3	0	4
Focal necrosis	0	1	0	0	0
Congestion	1	0	0	4	3
Heart: Mineralization	1	0	0	0	3
Myocarditis	1	1	1	0	0
Lung: Pneumoconiosis	4	3	1	1	2
Bronchitis	1	3	1	0	2
Esophagus:					
Ulceration	2	1	1	4	4
Lymphoid hyperplasia	2	1	3	3	1
Submucosal nematodes	0	5	2	1	1
Cysts	2	0	0	2	2
Proventriculus:					
Ulceration	3	2	2	2	1
Serosal granuloma	1	0	0	2	0
Proventriculitis	1	0	1	2	3
Intestinal flukes	1	2	2	0	4
Mesenteric granuloma	2	1	1	0	0
Cloaca: Hemorrhage	3	1	1	0	1
Lymphoid hyperplasia	2	0	3	1	2
Flukes	0	1	0	0	1
Kidney:					
Multifoc. interstit. nephritis	4	0	3	1	2
Flukes	1	0	0	0	2
Cystic tubules	0	2	3	0	1
Adrenal:					
Focal adrenalitis	1	1	1	1	0
Amyloidosis	0	0	1	0	1
Skeletal muscle:					
Sarcocysts	0	0	1	0	1
Hyaline fibers	0	1	1	0	1

The incidence of several conditions varied significantly between target sites at Tacoma, Shelton, and Seattle compared to reference sites at Smith and Goose Island. The incidence of tapeworm and traumatic lesions was significantly higher at the target sites than the reference areas (chi-square, $p<.05$). Liver weights also varied significantly by site (ANOVA, $p<.001$) though the two target sites at Seattle and Tacoma had the highest and lowest liver weights, respectively. A detailed description of different conditions is provided below.

Parasites. Almost every bird examined had at least two types of parasites in the alimentary tract. At least two varieties of nematodes were found free in the lumen or attached to the mucosa of the esophagus and crop, and other burrowing nematodes infested the submucosa of the esophagus, gizzard, and mesentaries. Nematodes of the genus Tetrameres, which encapsulate within the gastric glands of the proventriculus, were present in about 30% of the gulls. Birds from all sites except Seattle had infestations of Tetrameres. Forty-three percent of the birds from Goose and Smith Islands were exposed, as were about 30% of those from Shelton and Tacoma. No Tetrameres were identified in birds from Seattle, although tracts of unidentified parasites were present in the proventriculus of some Seattle birds.

Most of the birds from each breeding colony (except Goose and Smith Islands) had tapeworm infestations. Individual birds had severe parasitism, with the small bowel almost completely blocked by tapeworms. In severe cases the scolex of some worms almost perforated the intestinal wall.

A high percentage of birds at Tacoma and Shelton were infested with tapeworms, while those from Seattle and Smith Island had higher infestations of esophageal nematodes. Differences probably reflect foraging opportunities in the vicinity of the colonies and differences in parasite populations along the coast compared to the open parts of the Sound and the inland arms. Birds from Goose Island had somewhat lower infestations, but the overall incidence was high for all types of parasites, indicating that the prey base of all of the gulls supports parasites. Infestations of intestinal flukes were common in birds from all colonies except Seattle.

Traumatic lesions. High frequencies of traumatic lesions (generally, injuries to the skin or feet) were not seen in the majority of the populations of breeding females (Table 20). This low incidence may be a reflection of the general body condition of the birds, in that birds in poor condition as a result of injuries would be less likely to have been incubating three egg clutches. One bird was clearly the exception, and perhaps an indication of how stalwart gulls are, as she had a chicken femur (probably from foraging at a garbage dump) penetrating through the proventriculus wall, the peritoneum and body wall, into the musculature of the thigh. The bone was almost completely encapsulated, indicating that it was an old injury, but continuing damage was occurring, as there was considerable ulceration of the gizzard lining from the persistent abrasion of the end of the bone. This bird, it must be remembered, was a successful breeding female who had just laid three eggs, and otherwise appeared in good condition.

Two sites had birds with a high incidence of traumatic lesions, the Shelton site on the grounds of the Simpson Timber Co., and the colony at St. Regis Paper Co., Tacoma, where many birds had ulcers or traumatic lesions on the feet. The exposure to caustics at the Tacoma site was apparent from foot lesions, including severely inflamed synovial capsules at the heel joints of several birds, fresh or healed ulcers in the webs or on the toes of some, and one broken toe. Gulls from the lumber company site also had many minor fresh and healed ulcers in the webs and toes, apparently caused by splinters. None of the birds appeared debilitated by the minor toe and web injuries.

Abrasions and erosion of the mucosa of the esophagus and hemorrhage of the koilin lining of the gizzard was a common occurrence in birds from all colonies, undoubtedly resulting from ingestion of shells, stones, glass, nails, bones, plastic, and metal fragments (all of which were recovered from the gizzards of gulls).

Two birds were observed to have flacid paralysis of the crop, one with ulcerative lesions caused by a metal fragment (spiral shaped lathe turning) in the gizzard, and the other with hemorrhagic ulceration of the esophageal lining. Crop paralysis is consistent with heavy metal exposure and toxicity, but subtle changes of liver or kidney cells which would confirm heavy metal involvement could not be observed in either bird because of autolysis of the tissue prior to necropsy.

Dust particulates were present in the lungs of many birds (pneumoconiosis), especially from the colonies at Tacoma and Goose and Smith Islands. Pneumoconiosis is characterized by small fibrotic nodules containing crystalline particulates, often associated with lymphocytic nodules or diffuse lymphocytic reaction adjacent to the secondary bronchi within the lung. Burrowing birds and birds breeding in dusty environments would be expected to develop low grade pneumoconiosis. Goose and Smith Islands must be exposed to offshore winds and blowing sand and dust with some regularity. Inhalation of dust by Tacoma birds is probably correlated with exposure to dust from the paper mill. One might expect sawdust from the timber company to contribute to pneumoconiosis, but the particle size of sawdust is apparently too large or too hygroscopic to penetrate deep into airways.

Infectious agents. Most birds in the study appeared at gross necropsy to be relatively free of acute bacterial infections, although enlarged spleens in 7 birds and reactive spleens in 6 of the 37 breeding females indicated acute or chronic disease (Tables 20 and 21). There was a very high incidence of mild multifocal lymphocytic hepatitis (liver inflammation) in birds from all colonies, indicating a chronic, low-level of infection from the gastro-intestinal tract (probably food habit or parasite related). Similar incidences of mild multifocal interstitial nephritis (kidney inflammation) and renal, hepatic and adrenal amyloidosis (accumulations of gylcoprotein deposits as secondary reaction to degenerative processes) reflect chronic exposure to bacteria, toxicants, or parasites.

The most obvious examples of virulent infections were in two birds with yolk peritonitis, as a result of ovulation into the body cavity and consequent inflammation. Both of these were probably secondary to salpingitis (inflammation of the oviduct), which may have been responsible

for the failure of the infundibulum to collect the yolk at the time of ovulation. The oviduct infection probably spread into the peritoneal cavity after rupture of the yolk. The incidence of this problem is difficult to quantify, as these two birds were clearly in distress and therefore obvious to the collectors, who were looking for sick birds. Both of these birds would almost certainly have been excluded from the study of breeding females, as neither would have been incubating three egg clutches, having lost an egg internally.

Four gulls were discovered to have mesenteric granulomas on membranes surrounding the viscera. Two of these birds were from the Goose Island colony and one each was from the Shelton and Tacoma colonies. Fungal hyphae were observed in some of the nodules in one of the Goose Island birds, but no others exhibited an etiological agent. All of the granulomas may have been of fungal origin, but some could have been nematode induced.

Cloacal and urinary tract infections or inflammation were infrequently present in birds from every site. The etiology of the unusual cloacal inflammation with lymphoid hyperplasia in three birds from Shelton is unknown. Kidney inflammation was common at Goose Island, Smith Island and Shelton with birds exhibiting multifocal interstitial nephritis and renal amyloidosis. Two birds from Tacoma also had amyloid deposits in the kidney probably indicating previous chronic infection or possibly exposure to pollutants.

Liver pathology. The liver is a particularly good indicator of the overall health of a bird, and a good indicator of exposure to toxicants. Gulls in this study had marked variations in the size of their livers, which varied from 21.3 g to 52.0 g (244% variation) in birds whose total body weight varied less than 20%. A number of hypotheses may be put forward to propose an explanation, but without additional data the final causes of the marked variation cannot be explained.

Average liver weights and range of weights are presented in Table 22 and show considerable variation between sites. All birds examined from Seattle had large livers, with weights ranging from 42.8 g to 52.0 g (47.1 g average or 50.8 g/kg body wt.). All birds from Tacoma had small livers, ranging from 24.6 to 28.7 g (28.8 g average or 30.3 g/kg body wt.) The other three sites were mixed, having some birds with large and some with small livers. Complete data on liver weights of Glaucous-winged Gulls from other areas are not available, but values of juvenile Herring Gulls of nearly adult weight are given by Miller et al. (1978). Control livers averaged 26 g/kg body weight, with increases to 42 g/kg for birds exposed to South Louisiana crude oil. Younger gulls reported by Peakall et al. (1982) had proportionally larger livers, averaging 44.6 g/kg for controls.

Enlargement of the liver can occur with induction of liver enzymes. Pollutants which induce mixed-function oxidases (MFO) cause hypertrophy of hepatocytes and overall enlargement of the liver, accompanied by a large increase in the MFO and cytochrome activity (Miller et al., 1978; Peakall et al., 1982; Gorsline et al., 1981; Patton and Dieter, 1979; Szaro et al., 1978). Enlarged livers and elevated porphyrin levels correlated to chlorinated hydrocarbon residues has been reported in Herring Gull embryos from Lake Ontario (Gilbertson and Fox, 1977). Hypertrophy can often be quantified by morphometric measurements of hepatocytes, but has not been

Table 22. Liver weights of Glaucous-winged Gulls from different sites in western Washington. Site values differ significantly by ANOVA ($p<.001$). Birds from Seattle have significantly heavier livers than those from every other site ($p<.05$ for all cases).

Site	n	Mean	Std. dev	# > 40 g
Target sites				
Seattle	6	47.1	4.1	6
Tacoma	9	28.8	4.0	0
Shelton	12	33.5	7.4	2
Reference sites				
Goose Is.	9	31.3	9.0	2
Smith Is.	8	33.4	8.8	3

carried out in this study. Pollutant induced hypertrophy would normally be expected to be accompanied by some indicators of toxicity within the liver, including fatty changes, iron deposits, and pollutant residues. Only one of the 13 livers in excess of 40g had iron deposits and none showed fatty changes.

In severe cases of liver damage, the liver may atrophy. Therefore, an additional possible hypothesis is that many of the birds in this study have liver atrophy associated with hepatocellular damage. Sixteen of the 36 birds collected had livers of 30 g or less, and were a majority of the birds at Goose Island and Tacoma. Most of the birds from Shelton and Smith Island had livers of 30-40 g. The overall health of the birds appeared good, therefore it is unlikely that the disparity in liver size is due to liver atrophy.

The simplest explanation for the variation in liver weight would be that there is great variation in liver size in gulls. To our knowledge, variation in gull liver weights has not been reported. The variations in reported liver weight of Herring Gulls collected for pollution studies by the Canadian Wildlife Service are quite small (Miller et al., 1978; Peakall et al., 1982).

Another, perhaps more attractive hypothesis, is that the wide hybridization between Western and Glaucous-winged Gulls has resulted in large variation in liver size. The liver sizes of California Western Gulls and Alaska Glaucous-winged Gulls are not in the accessible literature. It is possible that the two species have quite different liver sizes, and the birds in the hybrid zone (Washington) could be highly variable, though the geographic pattern in liver weights is not consistent with the suspected degree of hybridization at our study sites. The plumage of all gulls has been saved, which might allow a determination of the hybrid status of each individual.

Five additional histological parameters of the liver can be used as indicators of health and/or pollutant exposure: hepatitis, fatty change, amyloidosis, focal necrosis, and hepatocellular iron. Table 21 shows the incidence of each of these along with congestion, which can often be an artifactual result of the cause of death which results in the retention of much blood in the liver. Diffuse hepatitis is infection or inflammation of many liver cells, resulting from disease exposure or toxicosis. Fatty changes often accompany degenerative processes but may be physiologic in origin in female birds during the breeding season when yolk lipids and proteins are being synthesized. Amyloid deposits are accumulations of glycoprotein (derived from immunoglobulins) within cells often occurring secondarily to degenerative or infectious processes. Amyloid may remain for long periods and be indicative of chronic or previous severe liver damage. Focal necrosis, which was observed in only one bird from Tacoma, is an indication of an acute local liver infection. Hepatocellular iron accumulations are an indication of liver damage or disease. Hepatocellular damage from toxicants frequently causes accumulations of iron within both hepatocytes and Kupfer's cells (reticulo-endothelial cells responsible for phagocytosis of damaged cells and debris). Hepatocytes stain positive for iron when damaged, and Kupfer Cells stain positive under a number of conditions such as hemolytic anemia, bacterial infections, blood parasites, bleeding into the bowel, or exposure to toxins. The birds with the largest

livers, however, generally stained negative with Prussian Blue, an iron-positive stain. If the largest livers were the result of hepatocellular hypertrophy, one would expect other symptoms of pollutant exposure. No liver or renal lesions reflective of heavy metal exposure were observed such as those described by Hoffman et al. (1981) which consisted of renal degeneration with some hepatic and cardiac necrosis.

Birds from Goose and Smith Islands and Tacoma had liver hepatocytes which were the most prominently stained with Prussian Blue. All birds from Tacoma had small livers and were the most Prussian Blue positive. The correlation of incidence of positive Prussian Blue staining and small livers is very significant, with 14 of 15 iron-positive livers being less than 30 g. Most of the birds with small, iron-positive livers also showed fatty changes and mild diffuse hepatitis. The combination of the three parameters indicates exposure to some insult causing liver damage in the Tacoma colony and the two relatively pristine colonies at Smith and Goose Islands.

The birds breeding at Shelton had variable incidence of hepatitis, fatty changes, hepatocellular iron, and amyloid deposits. The occurrences of each were not correlated, with each affected bird having only one or two of the conditions. None of the birds at Shelton appeared abnormal in any way.

In summary, the birds collected in Seattle present the most confusing situation. All birds had very large livers, nearly twice the size of birds from Tacoma, and all were without significant liver pathology. The two most plausible explanations are: local variation in normal, healthy liver size; or chronic exposure to hydrocarbon or organochlorine pollutants which have induced liver enzymes and hypertrophy in a population of birds which is showing excellent accommodation to the pollutants. Residue analysis should be performed on these livers to resolve the question.

Right oviducts. In gulls as in most other avian groups the female reproductive tract is asymmetrical. Gonads and primordia and ducts are symmetrically present early in development but differentiation occurs only on the left side, with the right ovarian and oviducial primordia regressing. Adult females normally do not have a right oviduct although some normal individuals have a small (5 mm long or less) residual diverticulum extending from the right side of the cloaca. The right ovary if present is not functional and if induced to develop by removal of the left gonad and application of the appropriate hormonal stimulus, will develop into an ovotestis.

Right oviducts fail to completely regress under conditions of exogenous estrogenic hormone application during embryogenesis. Injection of steroid hormones or hormone analogs (estradiol, diethyl estilbestrol (DES), o,p-DDT, kepone, or methoxychlor, for example) partially prevent the regression of the right Mullerian duct resulting in the presence of an enlarged variably sized incomplete right oviduct (Romanoff, 1960; Fry and Toone, 1981).

The incidence and size of right oviducts observed in birds in this study varied with colony location. Most birds from Goose Island (5 of 6) and Seattle (5 of 6) had completely regressed or small right oviducts (less than 5 mm); this is the expected normal development. All birds from Tacoma

(6 of 6) had right oviducts larger than 10 mm. Birds from Smith Island and Shelton were intermediate in both frequency and size of right oviduct with a high incidence of right oviducts (Smith - 5 of 6, Shelton - 6 of 7) but a lower proportion than Tacoma of large right oviducts (Smith - 3 of 6, Shelton - 2 of 7).

There is no information available concerning the incidence and size of right oviducts in wild gull populations. Additionally there is no information correlating embryonic estrogenic exposure and the presence of right oviducts in adult gulls. Most work has been done with poultry species and the work of Fry and Toone (1981) examines only gull chicks at the time of hatching. A more complete correlation of historical organochlorine exposure of birds at each of the colony sites would be valuable.

5.2.8 Mortality

Between one and five dead gulls were seen at each of the five study sites. There was no indication of higher adult mortality at the target sites compared to reference sites during the breeding season.

Dead nestlings and eggs were found at all sites during this study. The rates of mortality for nestlings and eggs are reflected in the tables showing hatching success and fledging success (Table 15 and Section 5.2.3). The cause of death of eggs and young was not specifically determined due to the infrequent visits to nests and the interval between visits. However, at all sites most egg and young mortality was apparently related to displacement of eggs and young during territorial disputes between adults, exposing the eggs and young to attack by non-parent adults.

5.2.9 Foods

There were limited observations of foods eaten by nestlings. The food items were classified into general categories: fish, bivalves, and processed foods (bread, chicken, hot dogs, ham, etc.). Most observations were of foods regurgitated by nestlings during handling. There were seven observations of food in the Smith Island colony, all fish. There were seven observations of food in the Seattle colony, six were fish and one was processed food (french fries). There were eight observations in the Tacoma colony, six were fish and two were processed foods (bread and chicken). There were eight observations in the Olympia colony, all of fish. There were eleven observations in the Shelton colony, one of fish, three of processed foods (green peppers, tomatoes, bread, cheese, ham), one of clams and fish, and six of clams. There were only three observations of foods in the Goose Island colony, two of fish and one frog (Rana sp.).

5.3 Conclusions

Comparison of numbers of breeding Glaucous-winged Gulls counted at our study sites in 1984 with historical information reveals gull populations appear to be increasing throughout the Puget Sound area. Data on clutch size, hatching success, fledging success, and growth rates of young showed variations between sites but generally, all colonies appeared healthy and without major reproductive problems. A high incidence of supernormal

clutches that was documented at the Seattle gull colony in the 1970s was not observed in 1984. Gull colonies at Seattle, Tacoma, and Shelton, our target or "most contaminated" sites, had mean (1984) eggshell thickness measurements that were significantly thinner than eggs collected in the San Juan Islands (reference values) before 1947. The degree of eggshell thinning at these three gull colonies relative to the pre-1947 reference values ranged from 8-10%. Mean eggshell thicknesses at the two control sites, Smith Island and Goose Island, were not significantly different from the pre-1947 reference egg measurements. These comparisons are complicated by the hybrid status of some of the gulls in our study areas and the lack of historical eggshell thickness measurements for Puget Sound.

The extent of pathology in the general population is extremely difficult to assess, but the gulls we sampled exhibited a variety of lesions and variability in liver weight, indicating a possible significant exposure to disease agents and/or pollutants. We cannot determine if gulls are particularly resilient and are thereby able to accommodate significant toxic exposure through liver hypertrophy with only minimal observable damage to the liver and kidneys (as evidenced by hepatitis, nephritis, and amyloid deposits in liver, kidney, and adrenals).

A true indication of the health of the entire population is extremely difficult to make, because of the many biases in sampling which are inherent in any sampling protocol. Much care should be exercised in interpreting the results of this study with respect to the extent of damage to birds, as only the healthiest birds in the population were systematically collected. The incidence of pathology in the population as a whole may be higher than the incidence we found for breeding females.

Several conditions occurred in a geographical pattern suggesting a possible relationship with pollutants. These included enlarged livers and possible liver atrophy, the incidence of traumatic lesions, and the degree of eggshell thinning. Additional tests including chemical analysis of collected tissues will be required to further explore the possible relationship between these conditions and environmental contaminants.

6. GREAT BLUE HERON

Great Blue Herons breed throughout western Washington. They feed in shallow marine waters as well as in freshwater and upland areas. In the Puget Sound area, some breeding colonies (heronries) are located adjacent to marine waters. The nests in these heronries are built 15-30 meters high in trees of various species.

A number of studies throughout North America and in Europe have documented pollutant-related biological effects in herons. Major pollutant-related effects documented for herons include: 1) mortality of adults and young, related to tissue residues of DDE, DDT, dieldrin, endrin, or mercury (Ohlendorf et al., 1981; Faber et al., 1972; Prestt, 1970; Call et al., 1976; Van der Molen et al., 1982); 2) abnormal behavior (egg breaking) by adults, related to residues of DDE, DDT, or dieldrin in eggs (Cooke et al., 1976; Konermann et al., 1978; Prestt, 1970); 3) eggshell thinning, related to DDE residues in eggs (Faber and Hickey, 1973; King et al., 1978; Ohlendorf et al., 1979; Blus et al., 1980; Laporte, 1982; Cooke et al., 1976; Ohlendorf et al., 1978; and Henny et al., 1984); 4) congenital abnormality (crossed bill), suspected to be related to high residues of chlorinated hydrocarbons (Gilbertson et al., 1976); and 5) reduced clutch size and productivity, related to residues of DDE in eggs (Henny et al., 1984; Konermann et al., 1978).

Research was conducted at nesting sites where adult Great Blue Herons were likely to be feeding wholly or partially in the near-shore marine waters of Puget Sound. Observations on foraging sites and prey were used to examine the link between the herons and the marine environment. Evidence for pollutant-related biological disorders was evaluated by gathering data on population status, mortality, and reproductive success at the heronries. Samples were collected or scavenged for eggshell measurements, prey identification, and future chemical analyses. We compared measurements of reproductive success and eggshell thickness between the sites near heavy industrialization, intensive agricultural activity, and in relatively undeveloped areas. If pollutants were related to significant problems in the reproductive health of herons in the Puget Sound area, we would expect to find differences in the reproductive success and eggshell thickness measurements at heronries located in the different areas.

6.1 Methods

6.1.1 Study site locations and description

The locations of the eight heronries studied in western Washington are shown in Figure 13. Four of these eight heronries were our primary study sites (West Seattle, Dumas Bay, Nisqually, and Totten Inlet). Three heronries were studied less intensively (Auburn, March Point, Long Island), and data from the Samish Island heronry were obtained largely through the cooperation of Toby Michelina of Western Washington University.

Heronries located at West Seattle and Dumas Bay were near the heavily industrialized areas of Elliott Bay and Commencement Bay. Chemical contaminants are known to occur at relatively high concentrations in these

industrialized bays (Long, 1982). Intensive agricultural activity is the dominant land use inland from the heronries at Samish Island and March Point. The Nisqually, Totten Inlet, and Long Island heronries are located near relatively undeveloped areas.

With the exception of the Auburn site, all of the heronries were located near marine waters where the herons were suspected to forage predominantly in a shallow marine or estuarine environment. Table 23 details the species of trees where nests were found and range in the nest heights for each of the heronries studied.

6.1.2 Observation effort

Table 24 shows the observation effort for the eight heronries investigated. A total of 119 visits involving 217 hours were made from February to August 1984. Blinds which were located in a tree adjacent to the heronry were utilized to observe a limited number of nests at the Nisqually heronry and the Samish Island heronry. Observations at the March Point, West Seattle, Dumas Bay, Auburn, Totten Inlet, and Long Island heronries were made from the ground. Generally, each visit to the heronry was limited to two hours or less to minimize disturbance of the nesting herons.

6.1.3 Nest censuses and nest contents surveys

In January and February 1984, the number of old nests present in each of eight heronries was determined by counting from below the nest trees. These initial nest counts were conducted before the herons began nesting activity, and the nests were highly visible because the deciduous trees had yet to leaf out. To facilitate surveys for young in nests throughout the breeding season, all nest trees at five of the heronries (Samish Island, West Seattle, Dumas Bay, Nisqually, and Totten Inlet) were tagged with flagging tape and the individual nests were numbered on these tags. Due to the topography of the Auburn heronry site, young in nests were readily observed from a nearby vantage point; individual nests at this site were monitored by reference to a sketch of the nest locations. Nests at the March Point and Long Island heronries were counted, but not monitored throughout the breeding season.

At six heronries individual nests were surveyed a minimum of once a week after hatching had begun. The number of nests surveyed at each site varied due to the number of nests present, and the ability to view nests from the ground or from a tree-blind as the nest trees became fully leafed.

A nest was considered active if adults or young were seen on the nest or if droppings were conspicuous on or below the nest. Numbers and approximate sizes of young in nests were recorded. To determine fledging success, all young herons that were seen six to eight weeks after their estimated time of hatching were considered fledged. Nests were considered successful if at least one young fledged.

6.1.4 Scavenging under nests

The ground below nest trees was searched frequently on visits to four heronries (West Seattle, Dumas Bay, Nisqually, and Totten Inlet). An attempt was made to locate any dead adults or nestlings, prey items, or

Table 23. Descriptions of trees in which Great Blue Heron nests were found and nest heights at eight sites in western Washington, 1984.

Site	Tree species	Approximate range in nest heights (m)
Samish Island	Cottonwood/red alder	23–30
March Point	Red alder/big-leaf maple/ douglas fir/western red cedar	12–23
West Seattle	Big-leaf maple	20–25
Dumas Bay	Red alder	18–24
Auburn	Red alder	15–24
Nisqually	Red alder	15–27
Totten Inlet	Red alder	18–30
Long Island	Western hemlock/sitka spruce/ western red cedar	23–35

Table 24. Observation effort at eight heronries in western Washington, February - August 1984.

Site	No. days visited	No. hours observed
Samish Island *	45	110
March Point	4	4
West Seattle	25	26
Dumas Bay	9	13
Auburn	7	7
Nisqually	19	43
Totten Inlet	8	11
Long Island	2	3
Total	119	217

* Observations reported by Toby Michelina, Western Washington University

portions of eggshells. Portions of hatched eggshells (eggshell fragments) were collected for eggshell thickness measurements. Only large eggshell fragments (>= one-half the size of a whole eggshell) were collected. The number of hatched eggshells was compared to examine hatching times between locations as reported by Werschkul et al. (1977) and Bayer and McMahon (1981). The total number of ground searches varied somewhat between sites, but generally occurred a minimum of once a week between the beginning of hatching and the fledging of the young. Ground searches lasted from 15-45 minutes depending on the number of nest trees in the heronry and the density of the understory vegetation.

In addition to the ground searches at the four heronries noted above, hatched eggshells were scavenged opportunistically during visits to four other heronries and T. Michelina reported data on prey items found below nest trees of the heronry at Samish Island.

6.1.5 Observations of adults

At six heronries, opportunistic observations were made of adult herons to determine flight paths to and from the heronries and to identify prey brought to the young. Suspected foraging sites near some of the heronries were examined to document the presence or absence of adult herons.

6.1.6 Observations for abnormalities

Adult and nestling herons were examined for gross external abnormalities while conducting nest surveys, although only the head and neck of each heron were commonly observed. The 61 dead nestlings found during the ground searches of the heronries were examined for the presence of external abnormalities. Finally, five to six clutches of eggs at each of six heronries were examined for obvious external abnormalities.

6.1.7 Collection of eggs

Fresh, whole eggs were collected from six heronries. One egg was collected from a clutch of eggs in each of five nests at the Samish Island, March Point, Nisqually, and Totten Inlet colonies, while one egg was collected from a clutch of eggs in each of six nests at the Dumas Bay and West Seattle heronries. The clutch size in each nest (and any nearby nests) was noted while collecting these eggs.

Collected eggs were wrapped in aluminum foil and stored in a refrigerator until blown into chemically clean (methylene chloride-rinsed) glass jars with aluminum foil liners. The blown eggshells were washed out and allowed to dry.

6.1.8 Measurements of eggshells and eggshell fragments

The eggshell thickness of all whole eggs collected and eggshell fragments salvaged was determined. See Glaucous-winged Gull methods for details (Section 5.1.11).

Three whole eggs which were measured for thickness only at one end of the egg, instead of at the waist, were not included in our whole egg sample because their thicknesses were significantly lower than the waist-thickness

measurements of all other whole eggs (t-test, $p<.05$). Of the 330 eggshell fragments collected, 222 had eggshell membranes intact, while 108 had missing shell membranes. For the analyses of eggshell fragments we corrected the thickness measurements for the fragments without membranes by adding 0.0675 mm, the average thickness of 20 membranes that were separated from eggshell fragments and measured individually. There was no significant difference between the thickness means of eggshell fragments with membranes intact and fragments without membranes which were corrected by adding 0.0675 mm (t-test, $p>.05$).

Heron eggshell thicknesses from this study were compared to the pre-1947 values reported for the "Pacific Northwest" by Anderson and Hickey (1972). Percent eggshell thinning was calculated using the Anderson and Hickey (1972) values as a base.

6.2 Results and Discussion

6.2.1 Population status and changes over time

Observations on overall numbers of nests in the eight heronries examined during 1984 were compared to historical data located on these individual heronries (Table 25). Based on the limited historical data which was available, we found no evidence that these heronries were experiencing significant declines in nest numbers over time.

No historical data on nest numbers were available for the March Point and West Seattle heronries. The numbers of nesting herons at Samish Island, Dumas Bay, Nisqually, and Totten Inlet have apparently increased over time, while nest numbers at the Auburn and Long Island sites have fluctuated, but appear to be stable or possibly increasing.

6.2.2 Hatching synchrony

Figure 15 presents the results of the hatching synchrony data from the heronries at West Seattle, Dumas Bay, Nisqually, and Totten Inlet. These heronries are located relatively close to one another, the greatest distance between any two of the four heronries was approximately 120 km (see Figure 13).

At the West Seattle heronry most of the hatched eggshells were found in early June while at the Dumas Bay, Nisqually, and Totten Inlet heronries most hatched eggshells were found approximately a month earlier, in late April or early May. In addition, the first observation of young in nests at the West Seattle heronry was on 2 June, more than a month later than the first observations of young at the other three heronries.

We were unable to determine the cause of the late breeding at the West Seattle heronry. Peak hatching times reported for more than 10 heronries on the Oregon coast occurred in late April or early May, similar to the peak hatching times we found at the Dumas Bay, Nisqually, and the Totten Inlet heronries (Werschkul et al., 1977; Bayer and McMahon, 1981). Weather was apparently not a factor in the late breeding at the West Seattle heronry because the four heronries we examined for hatching chronology were exposed to the same weather patterns. The late breeding at the West

Table 25. Comparison of 1984 and historical data for total nest numbers in eight heronries in western Washington.

	Total number nests 1984 (this study)	Historical data number nests-year	Historical data sources
Samish Island	334	>50, 1925	J. Edson, unpubl. notes
March Point	>42	No data	-----
West Seattle	16	Colony in area since 1940's	Conversation with local resident
Dumas Bay	46	Colony in area since 1940's; 5, 1978; 7, 1979;, 24, 1981; 33, 1982	Conversation with local resident; Tacoma Audubon Society; Wash. Dept. Game
Auburn	14	First nests, 1968; 15, 1981; 18, 1982; 8 (active) 1983	Tacoma Audubon Society; Wash. Dept. Game; G. Alcorn, pers. comm.
Nisqually	53	First nests observed, 1977	Unpubl. obs., The Evergreen State Coll.
Totten Inlet	75	30, 1978; 55, 1983	Wash. Dept. Game
Long Island	128	125, 1981; 110, 1982; 93, 1983	U. Wilson, pers. comm.

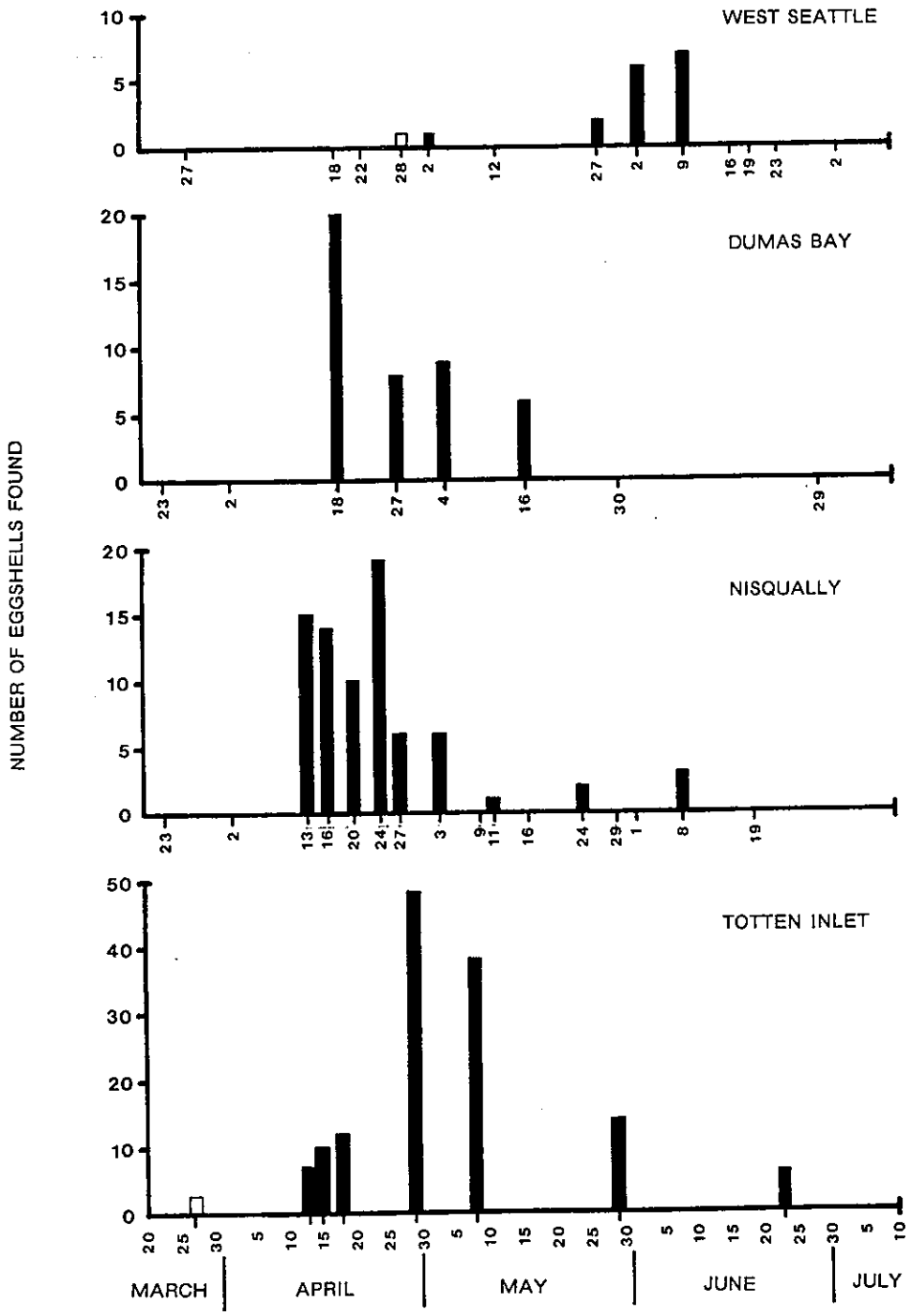

Figure 15. Dates that eggshells from hatched eggs were found at four Great Blue Heron nesting areas in western Washington, 1984. Unfilled bars represent eggshells that appeared weathered or were found prior to hatching at that site; these were presumed to be from the previous year. Dates of visits are marked by slashes below the x-axis.

Seattle heronry may be related to the small number of herons (32, see Table 25) nesting there (less than half the number nesting at the other three study sites). Werschkul et al. (1977) found smaller heronries had later arrival times and hatching dates than larger heronries.

6.2.3 Prey and foraging sites

Data on foraging sites and Great Blue Heron prey were gathered opportunistically during the ground searches under nest trees and while making observations to determine reproductive success at the heronries under investigation. These observations were made in order to determine if herons were feeding in marine areas and thus exposed to contaminants present in Puget Sound biota. Observations on foraging sites and prey for the individual heronries are detailed below.

Samish Island. T. Michelina reported that the herons at this large heronry feed in both Padilla and Samish Bays. Birds in the northern portions of the heronry apparently feed primarily in Samish Bay while birds in the southern portions of the heronry feed primarily in Padilla Bay. He also reported that prey found on the ground under nest trees consisted of approximately 50% flatfish, 30% an unidentified gunnel (fish) species, and 20% crab and small mammal remains.

March Point. Only four visits were made to this heronry located near Padilla Bay. On two visits in April regurgitated pellets consisting of the hair of small mammals were found below nest trees. No observations of herons flying to or from feeding areas were made.

West Seattle. Observations and ground searches at this small heronry were relatively frequent. Only one regurgitated pellet of small mammal hair was found below the nest trees along with one crab shell and the bones of four fish. Most observations of herons flying in and out of this heronry were to or from the area of Kellog Island, a small island near the mouth of the Duwamish River adjacent to Elliott Bay. The area surrounding Kellog Island is heavily industrialized. Eight to ten Great Blue Herons were seen feeding regularly at Kellog Island during the 1977 and 1978 breeding seasons, and approximately 30 birds were counted in the winter (Canning et al., 1979). They also noted herons flying from Kellog Island to the ridge where the heronry is located, but they did not locate the heronry.

Auburn. We searched the ground under the nest trees of this heronry once in April and once in May and found regurgitated pellets of small mammal hair and the remains of one large bullfrog (Rana catesbiana). The Tacoma Audubon Society chapter has observed this heronry over the last ten years and they report that the herons feed primarily on Townsends voles (Microtus townsendi) in the wet fields of the Auburn valley. We did not suspect or find evidence that the herons at this site were feeding in the marine waters of Puget Sound.

Dumas Bay. Ground searches at this heronry revealed regurgitated pellets of small mammal hair in March and April, and the remains of four fish in May. Herons were observed by us and reported by local residents to feed along the shoreline of Dumas Bay. The Tacoma Audubon Society chapter has also noted that up to 100 Great Blue Herons have been observed to

winter near the Hylebos Waterway of Commencement Bay, and they believe many of these herons may nest at nearby Dumas Bay. We observed Great Blue Herons feeding in the industrialized waterways of Commencement Bay during June, and saw three birds that flew directly from Commencement Bay toward the heronry at Dumas Bay.

Nisqually. Herons were observed feeding in the delta estuary, McAllister Creek, and the grasslands of the delta. The herons were believed to be feeding primarily in the estuary, as over 70% of 58 observations of heron flights were to or from the estuary. Prey was observed being fed to nestlings on eight occasions, seven of which were fish and one was a small mammal. Regurgitated pellets of small mammal hair were found on the ground searches during March and early April. One (unidentified) sculpin, five salmonids, and seven sandlance (Ammodytes hexapterus) were also found during the ground searches, along with one unidentified fish, remains of two large bullfrogs, and one Townsend's vole (Microtus townsendi).

Totten Inlet. Regurgitated pellets of small mammal hair were found below nest trees in March and early April. Three (unidentified) crab claws were found during May. This heronry is located near Totten Inlet, but we were unable to make observations of the inlet for foraging herons. On several visits herons were observed at a small freshwater marsh east of the heronry.

Great Blue Herons were observed to feed in freshwater and upland areas as well as shallow marine waters. The extent that herons are feeding in these different areas may largely determine their intake of pollutants via prey utilized in these different areas. The limited data we collected indicate that birds at the majority of heronries investigated were utilizing the shallow marine waters of Puget Sound for feeding during the breeding season. However, these observations also reveal that freshwater and/or upland areas are also utilized for feeding by herons nesting near Puget Sound.

6.2.4 Mortality and abnormalities

No dead adult Great Blue Herons or eggshells that appeared broken by adults were located during our ground searches of heronries in western Washington. Observations of adult (minimum of 130) and nestling (minimum of 270) herons during nest surveys at five heronries (West Seattle, Dumas Bay, Nisqually, Totten Inlet, and Auburn) revealed no gross abnormalities of their heads or beaks. Whole fresh eggs observed during the course of the study appeared normal. A total of 61 dead nestling herons found below the nest trees at the Auburn (1), Dumas Bay (17), Nisqually (29), and Totten Inlet (14) heronries were examined for gross external abnormalities, and no abnormalities were noted. Differences between sites in numbers of dead nestlings found most likely reflect variation in search effort, type of understory, and presence of scavengers. We did not determine the cause of death of nestlings, although starvation was suspected to be the most likely cause. Starvation has been reported to be a major factor in nestling mortality of Great Blue Herons (Collazo, 1981). Thirty of the 61 dead nestlings found during the ground searches were in reasonably fresh condition, and these were frozen and stored for future chemical analyses.

Mortality of wild adult herons has been associated with high residues of DDE, DDT, dieldrin, and endrin in a number of investigations conducted in North America and Europe (Ohlendorf et al., 1981; Faber et al., 1972;, Prestt, 1970). In addition, high levels of DDE, dieldrin, and PCB in eggs have been linked to deliberate egg destruction by Grey Herons (Ardea cinerea) in England and by Black-crowned Night Herons (Nycticorax nycticorax) on Pigeon Island in eastern Lake Ontario (Prestt, 1970; Cooke et al., 1976; Price, 1976).

Gilbertson et al. (1976) reported finding a Black-crowned Night Heron chick with a crossed bill at Lake Ontario in 1973. Although they did not analyze chicks or eggs for contaminant levels, they noted that high PCB residues were found in eggs of gulls and terns from Lake Ontario in 1972 and 1973.

6.2.5 Reproductive success

We collected clutch size data from a minimum of five nests in each of the six heronries (Table 26). This information represents minimum clutch sizes only, as clutches were not observed repeatedly and some eggs could have been laid after our census.

The clutch sizes of a total of 33 successful nests from six heronries varied from 2 to 5. An analysis of variance of clutch sizes revealed that clutch size varied significantly by site (ANOVA, $p<.05$), with a significantly higher mean clutch size at the West Seattle heronry (t-test, $p<.05$) when compared to all other heronries except Dumas Bay. The mean clutch size for all six heronries combined was 3.7 (n=33), which is lower than has been reported for successfully reproducing heronries in western Oregon (4.19, n=32; Henny and Bethers, 1971) and in eastern Washington (4.45, n=20; Blus et al., 1980). However, because our data represent minimum clutch sizes only, clutch sizes may have been similar to the clutch sizes reported for successfully reproducing herons from other areas of the Pacific Northwest. T. Michelina reported a mean clutch size of 3.9 (s.d.=0.847, n=27) for the heronry at Samish Island in 1984. Henny et al. (1984) found that the clutch size and productivity of Black-crowned Night Herons from Oregon and Nevada (1978-1980) decreased when DDE levels in eggs exceeded 8 ppm (wet weight).

Information on fledging success of Great Blue Herons was obtained for five heronries, and is shown in Table 27. We consider these fledging success rates as minimum values because observations of nests located high in trees was often difficult, and there was no certainty that all young in a particular nest were observed.

The number of young fledged from a total of 94 successful nests in five heronries varied from 1 to 4. Analyses showed that average fledging success varied significantly by site (ANOVA, $p<.001$), with the Auburn heronry fledging higher numbers of young per successful nest than the other heronries investigated and the Totten Inlet heronry fledging lower numbers of young per successful nest than did the other heronries. However, lower numbers of fledglings per successful nest observed at the Totten Inlet heronry may have been the result of sampling difficulty, as this site was the most problematical to observe due to the topography of the site, height of the nests, and the dense foliage near nests.

Table 26. Clutch size of successful Great Blue Heron nests at six heronries in western Washington, 1984. Values are minimums since clutches were not followed repeatedly to confirm maximum clutch size.

Heronry	Total clutches observed	Number of eggs in each clutch				Clutch size mean (s.d.)
		2	3	4	5	
Samish Island	5	0	4	1	0	3.2 (.447)
March Point	5	0	4	1	0	3.2 (.447)
West Seattle	5	0	0	2	3	4.6 (.548)*
Dumas Bay	8	1	2	4	1	3.6 (.916)
Nisqually	5	0	1	4	0	3.8 (.447)
Totten Inlet	5	0	1	4	0	3.8 (.447)
All sites	33					3.7 (.728)

* Significantly higher mean clutch size than all other sites except Dumas Bay (t-test, $p<.05$).

Table 27. Fledging success of Great Blue Herons at five heronries in western Washington, 1984.

Heronry	Total successful nests observed	Number of young fledged per successful nest				Mean (s.d.)
		1	2	3	4	
West Seattle	8	1	5	2	0	2.1 (.64)
Dumas Bay	32	11	12	9	0	1.9 (.80)
Auburn	10	2	0	5	3	2.9 (1.1)
Nisqually	12	1	7	2	2	2.4 (.90)
Totten Inlet	32	18	9	5	0	1.6 (.76)
All sites	94					2.2 (.495)
All w/o Auburn*	84					2.0 (.345)

* All sites except the Auburn heronry - to distinguish sites where herons were known to be feeding at least partially in a marine or estuarine habitat.

The mean number of fledglings per successful nest for the four heronries where herons were known to be feeding at least partially in marine waters during the breeding season was 2.0 (n=84). This overall mean fledging rate for successful nests compares favorably to the 1.9 young per breeding pair reported by Henny (1972) to be necessary for the maintenance of a stable population of Great Blue Herons. The mean fledging rates per successful nest from these four heronries in western Washington are similar, though at the low end of the range compared to fledging rates reported in other studies of Great Blue Herons in the Pacific Northwest (Table 28). T. Michelina reported that the average number of young fledged per successful nest at the Samish Island heronry in 1984 was 1.9 (s.d.=0.583, n=24).

6.2.6 Eggshell thickness

Results of measurements from 32 whole eggs from six heronries and 330 eggshell fragments (greater than half the eggshell) from eight heronries are summarized in Table 29.

The overall mean thickness of hatched eggshell fragments from eight heronries was significantly greater than the mean thickness of whole eggs collected from six heronries (t-test, $p<.005$). However, because the number of whole eggs and eggshell fragments collected at individual sites varied considerably (Table 29), we employed a two-way ANOVA test to consider both the effects of 1) whole egg thickness versus eggshell fragment thickness and 2) the site variations. This analysis confirmed that eggshell fragments were significantly thicker than eggshells from whole eggs, even when the effects of site variations were considered (two-way ANOVA, $p<.05$). Similar findings have been reported by Bayer (1982) who measured the eggshell thickness of both hatched and unhatched eggshells of Great Blue Herons on the Oregon coast. He found that the mean thickness of hatched eggshells from all heronries he investigated (0.352, n=720) was significantly greater than the mean thickness of unhatched eggshells (0.332, n=41) (t-test, $p<.01$).

Our analysis of differences in eggshell thickness by individual heronry showed that the thickness of eggshell fragments varied significantly by site (ANOVA, $p<.025$), but site differences in eggshell thickness were not significant for whole eggs alone (ANOVA, $p>.05$). The small sample size for whole eggs was apparently not adequate to achieve significant differences between individual sites, despite the fact that mean whole egg thicknesses ranged from a low of 0.337 mm at March Point to a high of 0.379 at Dumas Bay. However, a two-way ANOVA did indicate significant site variations in eggshell thickness, even when the effect of differences between thicknesses of whole eggs and eggshell fragments were considered ($p<.05$).

Anderson and Hickey (1972) reported a mean thickness of 0.389 mm for whole Great Blue Heron eggs collected in the "Pacific Northwest" before 1947 (n=64). The overall mean thickness of whole eggs collected from six heronries in western Washington during 1984 was significantly thinner than this pre-1947 mean (Table 29, t-test, $p<.001$). On an individual site basis, whole eggs from Samish Island, March Point, West Seattle, and the Nisqually heronry were significantly thinner than the pre-1947 mean, while the mean thickness of whole eggs from the Dumas Bay and Totten Inlet

Table 28. Comparison of fledging success data for the Great Blue Heron in the Pacific Northwest.

Site	Year	No. nests sampled	Young fledged per successful nest	Source
W Oregon	1970	32	2.61	Henny and Bethers (1971)
W Oregon	1974	22-43	2.18-2.70	Werschkul et al. (1977)
NW Oregon	1975	27	2.32	English (1978)
N Idaho	1977	109	2.14	Collazo (1981)
N Idaho	1978	148	2.20	Collazo (1981)
E. Washington	1978	42	2.29-2.79	Blus et al. (1980)
W. Washington	1984	84	2.0	This study*

* Data from four heronries, excludes data from Auburn site (see Table 27).

Table 29. Great Blue Heron eggshell thickness from nine heronries in western Washington, 1984. Measurements of eggshell fragments that were missing membranes have been adjusted (see Section 6.1.8).

Heronry	Sample numbers		Eggshell thickness (mm) Mean (s.d.)		% change from pre-1947 thickness mean+	
	Whole eggs	Fragments	Whole eggs	Fragments	Whole eggs	Fragments
Samish Island	5	0	0.343 (.0215)	-	-12***	-
March Point	5	10	0.337 (.0381)	0.380 (.0425)	-13***	-2
West Seattle	5	18	0.368 (.0281)	0.381 (.0304)	-5*	-2
Dumas Bay	5	34	0.379 (.0389)	0.373 (.0244)	-3	-4***
Nisqually	5	78	0.363 (.0190)	0.387 (.0251)	-7**	-1
Totten Inlet	4	133	0.376 (.0262)	0.377 (.0318)	-3	-3**
Long Island	0	33	-	0.385 (.0288)	-	-1
Auburn	0	16	-	0.361 (.0452)	-	-7***
Henderson Inlet	0	8	-	0.393 (.0355)	-	+1
Mean for all sites			0.360 (.0314)	0.379 (.0309)	-7***	-2*

+ Pre-1947 whole egg thickness mean for "Pacific Northwest" Great Blue Herons was 0.389 mm (n=64), from Anderson and Hickey (1972).
Significance level, t-test, * = $p<.05$, ** = $p<.01$, *** = $p<.001$

heronries were thinner than the pre-1947 mean but the differences were not significant (Table 29). Percent change in the mean whole egg thickness compared to the pre-1947 mean ranged from a high of 12% and 13% at the Samish Island and March Point heronries, to 3% at the Dumas Bay and Totten Inlet heronries. The Samish and March Point heronries (the sites showing the greatest amount of eggshell thinning) are located near each other adjacent to Samish and Padilla Bays. Although these sites are unlikely locations of high industrial contamination, they would be exposed to run off from agricultural areas and possibly pesticides.

For eggshell fragments only, mean thickness for all heronries combined was significantly thinner than the pre-1947 whole egg mean (t-test, $p<.05$). On an individual site basis, eggshell fragments from Dumas Bay, Totten Inlet, and the Auburn heronry were significantly thinner than the pre-1947 whole egg mean, while eggshell fragments from March Point, West Seattle, Nisqually, and the Long Island heronry were thinner than the pre-1947 whole egg mean but the differences were not significant (Table 29). Eggshell fragments obtained from a heronry on Henderson Inlet in southern Puget Sound were slightly thicker than the pre-1947 whole egg mean, but this difference was not significant. T. Michelina measured 133 eggshell fragments recovered at the Samish Island heronry in 1984, and reported a mean thickness of 0.349 mm (s.d.=0.026).

DDE is the primary chemical contaminant reported to cause eggshell thinning in birds (Cooke, 1973). Herons and egrets are considered highly sensitive to DDE-induced eggshell thinning (Peakall, 1975). Eggshell thicknesses significantly lower than pre-1947 eggshell thicknesses have been reported for Great Blue Herons from throughout the United States and Canada (Table 30). Table 30 includes mean residues of DDE or total DDT in eggs and notes if a correlation between eggshell thinning and the DDE residues was determined.

6.3 Conclusions

Research was conducted on the biology and reproductive health of Great Blue Herons nesting in areas adjacent to Puget Sound in western Washington during 1984. For those heronries with historical data available, overall numbers of nests appeared to be increasing or stable. The hatching time at the West Seattle heronry occurred approximately one month later than three other heronries located within 120 km. Data on prey and foraging sites established that herons at the majority of the study sites near Puget Sound were using the shallow marine or estuarine waters for foraging. However, we also found evidence that herons at these sites were utilizing upland and/or freshwater habitats for feeding during the breeding season. No mortality of adult herons was discovered, and we found no eggs that appeared to be broken by abnormal adult behavior or as a result of eggshell thinning. Gross external abnormalities were not observed in adults, young, or eggs. Although some site differences in clutch size and fledging success were noted, we were unable to find patterns that distinguished the heronries near relatively contaminated areas from those located near relatively uncontaminated areas. Furthermore, all sites had fledging rates that were normal or near normal when compared to successfully reproducing heronries in other parts of the Pacific Northwest. Compared to pre-1947 measurements reported for the

Table 30. Comparison of eggshell thinning reported for Great Blue Herons (percent thinning compared to pre-1947 eggs). Decreases in eggshell thickness or thickness index are based on comparisons made in each study.

Year	State or location	No. sites	No. of samples	% decrease in Eggshell Thickness (ET) or Thickness Index (TI)*	DDE or total DDT level**	DDE correlation	Reference
1948-61	UT and TX	?	35	4.0 (TI)	-	ND	Anderson and Hickey (1972)
1956-59	WA and BC, Can.	?	9	9.0 (TI)	-	ND	Anderson and Hickey (1972)
1957-62	Ontario, Canada	?	24	6.0 (TI)	-	ND	Anderson and Hickey (1972)
1970	WI	1	5	16 (ET)	470 (1)	+	Faber and Hickey (1973)
1970	TX, Gulf Coast	?	20	13 (ET)	5.6 (w)	+	King et al. (1978)
1970	CA	1	59	10.4 (ET)	-	ND	Faber et al. (1972)
1972-73	U.S., Midwest	4	23	7.9 (ET)	2.2-18 (w)	+	Ohlendorf et al. (1979)
1972-73	FL	5	28	5.2 (ET)	0.46-12 (w)	+	Ohlendorf et al. (1979)
1975	OR	6	256	6.8-10.2 (ET)	-	ND	Bayer (1982)
1978	WA	1	8	13.1 (ET)	4.7 (w)	+	Blus et al. (1980)
1978	WA/OR	1	13	11.8 (ET)	3.3 (w)	+	Blus et al. (1980)
1978	TX, Gulf Coast	1	17	5.0 (ET)	3.7 (w)	ND	Mitchell et al. (1981)
1979	TX, Gulf Coast	1	16	0 (ET)	3.0 (w)	ND	Mitchell et al. (1981)
1979	Quebec, Canada	1	47	5.3 (ET)	2.4 (w)	+	Laporte (1982)
1984	WA, western	6	29	7.0 (ET)	-	ND	This study

* Thickness index is also known as the Ratcliffe index, derived by dividing the weight of the empty eggshell (in milligrams) by the product of the shell length (in millimeters) and the shell breadth.

** Mean value in ppm, weight basis - (w) = wet weight, (l) = lipid weight, (d) = dry weight

ND = not determined

"Pacific Northwest", significant eggshell thinning was documented at most of the heronries investigated. The degree of eggshell thinning exceeded 10% for whole eggs at two heronries (Samish Island and March Point) located in an agricultural area near Samish and Padilla Bays. These two heronries also had the lowest clutch sizes of any of our sites and the fledging rate at the Samish Island colony appeared to be below average. These results suggest that herons at Samish Island and March Point may be experiencing reproductive problems, possibly related to DDE-induced eggshell thinning. However, chemical analyses are needed to test this hypothesis.

7. PIGEON GUILLEMOT

Small groups of Pigeon Guillemots breed throughout the inland marine waters of Washington (Speich and Wahl, 1985), including along the Seattle and Tacoma waterfronts, among the most contaminated sites in Washington. This species is of particular interest because it obtains all of its food from marine food chains, feeding on a variety of organisms from throughout the water column, and it breeds and nests locally. Unlike the Glaucous-winged Gull and Great Blue Heron it is unable to feed in upland areas, and we know of no observations of its feeding in nonmarine waters. During the breeding season Pigeon Guillemots tend to forage relatively close to their nesting site, as compared to gulls and herons. Specificity to marine food chains and localized feeding make this species ideal for monitoring effects of local marine pollution and possible pollutant effects on population levels, reproduction, and the physiological health of individual birds. Other alcid species are known to accumulate various environmental pollutants (Ratcliffe, 1970; Fimreite et al., 1977; Ohlendorf, 1982). Alcids, including Pigeon Guillemots, do not appear to be highly susceptible to DDE-induced eggshell thinning (Ratcliffe, 1970; Fimreite et al., 1977), although one alcid species, the Common Murre (Uria aalge), from the Farallon Islands, California experienced 12% eggshell thinning correlated to DDE levels (Gress et al., 1971).

This study was designed to compare various measures of possible pollutant effects on Pigeon Guillemots from a reference site at Protection Island and the target sites in Hammersley Inlet and along the more contaminated waterfront areas at Seattle, Tacoma, and Olympia. For this purpose, eggshell thickness and other measures of reproductive success - clutch size, hatching success, fledging success, nestling growth rates, as well as incidences of gross pathology - were compared between sites.

7.1 Methods

7.1.1 Study site selection and locations

The study sites were chosen on the basis of their exposure to contaminants and proximity to urban and industrial areas (see Figure 13). Protection Island, north of Puget Sound, was chosen as a reference site because of its expected low levels of contaminants, its large number of breeding guillemots, and the past work done on guillemots there. The waterfronts of Olympia, Tacoma, and Seattle were chosen as the target sites. The results from these sites were pooled to accommodate the low guillemot numbers at each site (Wahl and Speich, 1984; Speich and Wahl, 1985). Hammersley Inlet, southern Puget Sound, was a site suspected to have an intermediate contaminant exposure. It contained a large number of accessible guillemot nests. Limited observations were also made at Eagle Harbor, a site of suspected high contaminant exposure.

7.1.2 Colony census

The censusing of Pigeon Guillemot nesting areas differed with each site. No attempt was made to census the Protection Island population because data from other investigators were available. The Pigeon Guillemots in Hammersley Inlet were censused from a boat 11 May and 4 June, when a count of all adults and all visible nesting holes was made. Censuses of adults along the Olympia waterfront were carried out 11 May, 23 May, and 1 July, and censuses for nests were made on 2 June, 4 June, 17 June, and 1 July. Counts of adults and searches for nests were conducted by boat on the Tacoma waterfront 11 June and 6 July, and included the shoreline from Pt. Defiance to Dumas Bay and parts of the south shore on Vashon and Maury Islands. Adults were counted and nest sites determined on the Seattle waterfront on four different dates. On 1 June adults were counted from various shore locations. The shoreline was searched by boat on 6 July, 17 July, and 24 July. The boat censuses covered the waterfront shoreline from Duwamish head to West Point, including the port waterways. Censuses of Eagle Harbor were made on 17 and 24 July.

7.1.3 Marking birds and nests

Nestlings and nests were marked to allow us to make repeated examinations of the same birds or nests. Nestlings in burrows were marked in two ways. When nestlings were small, marks were made on the webs of their feet with marking pens, allowing later identification. When the nestlings were larger, they were permanently marked with U.S. Fish & Wildlife Service serially numbered bird bands.

Nests were marked by three different methods. In Hammersley Inlet nests were marked by placing flagging tape with the nest number written on it near the burrow entrance. On the Olympia and Seattle waterfront nests were marked by spraying paint on the dock or pilings near the nest. On Protection Island the nests were marked by placing 30 or 50 cm long numbered stakes near the entrance. To reduce predation by resourceful crows (Corvus brachyrhynchos), the stakes in the Protection Island colony were placed a few meters away from the nest entrance, and relocation instructions were written for each study nest site.

7.1.4 Nest contents survey

Study nests on Protection Island were examined on each visit: 6 June, 12 June, 12 July, 19 July, 1 August, 8 August, and 29 August. Nests were found on only one trip to Seattle, 17 July, although searches for nests were also made 6 and 24 July. Nests were observed 15 June and 6 July on the Tacoma waterfront. In Hammersley Inlet, nests were examined on several trips: 4 June, 5 June, 18 June, 3 July, 25 July, and 28 July.

7.1.5 Collection of specimens

Six adult Pigeon Guillemots and their eggs were collected from both Protection Island and Hammersley Inlet, on the visits described above. It was not possible to determine the sex of the incubating birds from external observations or measurements, and not all the birds collected were females.

Adult Pigeon Guillemots, whole eggs, and egg fragments were collected from different sites for later examination:

Site	No. Adults	No. eggs	No. egg frgm.
Hammersley Inlet	6	19	3
Protection Is.	6	16	5
Seattle	0	4	0
Tacoma	0	1	0
Olympia	0	1	1

The adults were anesthetized with ether, and usually placed on ice, later placed in refrigeration, and a necropsy was performed as soon as possible, where tissue samples were taken. For specific details of the necropsy and histopathology procedure see Section 5.1.

All eggs collected and salvaged were later blown and the contents were placed in cold storage. All eggshell fragments are now in the Western Foundation of Vertebrate Zoology (WFVZ), Los Angeles and the whole egg shells are in the WFVZ and the Burke Memorial Washington State Museum, University of Washington, Seattle.

7.1.6 Reproductive success measurements

We were able to gather data for four measurements of reproductive success (clutch size, hatching success, fledging success, and nestling growth rates) only on Protection Island and in Hammersley Inlet. Clutch size was determined when nests were inspected, as was the number of eggs that hatched. Fledging success was inferred from the presence of large nestlings in burrows, and in some cases their absence on later visits. Growth rates were calculated by weighing nestlings on two or more visits. Weights only from the straight line portion of the growth curve were used.

7.1.7 Measurements of eggs

Eggshell thickness was determined for all eggshells and eggshell fragments obtained. All measurements were made by personnel of the WFVZ. For details see Glaucous-winged Gull methods (Section 5.1).

7.1.8 Observations of abnormalities

All eggs and nestlings handled were examined for abnormalities. The surface condition and shape of all eggs was observed. All the soft parts and the plumage of all nestlings handled were examined.

7.2 Results and Discussion

7.2.1 Population status

Census results of surveys of Pigeon Guillemots at our study sites are provided below:

Site	Date of high count	Max. # adults	# nests found	Comments
Hammersley In.	11 May	85	29	Incomplete count of nests
Olympia	11 May	30	4	Some adults from outside Olympia area
Tacoma	15 June	10-15	1	nest in Dumas Bay
Seattle	6 July	11	2	One nest had two clutches, more possible nests at West Pt.
Eagle Harbor	17 July	9	2	

Counts at Protection Island for 1984 were 1,834 birds (J. Galusha, pers. comm.). There are limited historical data from all sites except Protection Island for determining population trends. The limited records of sightings and collections at all sites except Protection Island at least indicates that Pigeon Guillemots did occur in these areas in previous years (Speich and Wahl, 1985). The more extensive data for Protection Island indicates the population has increased dramatically over the last few decades (Speich and Wahl, 1985).

7.2.2 Breeding chronology

Breeding chronology appeared normal at both Hammersley Inlet and Protection Island, the only sites where chronology could be examined. In both areas, most nests had eggs on our first nest searches in the beginning of June. At both sites a few eggs were found into late July. Unfortunately, we missed the start of egg laying, but it appears most eggs were laid in late May and early June, at both sites. The small number of visits does not allow for an accurate comparison of the times of egg laying between the two sites, or with other studies. The study by Hirsch (1981) on Protection Island in 1980 revealed a similar pattern of egg laying. Data from Mandarte Island (Drent et al., 1964) for 1957 through 1960 shows a shift of 1-2 weeks between years in the time of egg laying. However, the time period is similar to that that we observed.

7.2.3 Reproductive success

Four measures of reproductive success (clutch size, hatching rate, fledging success, and growth rate) were compared between sites and with values reported in the literature. For all measures of reproductive success except clutch size, the sample sizes at the target sites of Seattle, Tacoma, and Olympia were not large enough for meaningful analyses, thus precluding a comparison between target and reference areas.

Clutch size values for our study sites and values for Pigeon Guillemots reported in the literature were:

Site	Year	# nests	% nests with 2 eggs	Mean	Reference
Hammersley In.	1984	18	83	1.83	this study
Protection Is	1984	25	68	1.68	this study
Sea., Tac., & Oly.	1984	5	100	2.0	this study
Protection Is.	1979	35	100	2.0	Banks (1981)
Protection Is.	1980	31	49	1.48	Hirsch (1981)
Deception Pass	1957	42	79	1.8	Thorensen and Booth (1958)
Mandarte Is.	1957-60	162	91	1.91	Drent et al. (1965)

There were no significant differences in mean clutch size between our three study areas (ANOVA, $p>.05$) and clutch sizes for all our study areas, including the limited sample from the pooled target areas in Seattle, Tacoma, and Olympia, appeared normal.

Hatching rates (percent of eggs laid that hatched) and fledging rates (percent of eggs laid that yielded fledged young) for guillemots appeared low at some of our study sites compared to other areas. Values for our study areas and those reported in the literature were:

Site	Year	# eggs	% hatch.	% fledg.	Reference
Hammersley In.	1984	12	83	83	this study
Seattle	1984	6	33	-	this study
Protection Is.	1984	26	50	38	this study
Protection Is.	1980	-	91	61	Hirsch (1981)
Mandarte Is.	1957-60	-	62	-	Drent et al. (1965)

The limited hatching rate data from Seattle though based on a very small sample was well below the hatching rate at other sites and reported in the literature. The fledging rate at Protection Island also appeared to be low in 1984 compared to that for 1980 and differed significantly from the fledging rate at Hammersley Inlet (chi-square, $p<.01$).

Growth rates for nestlings were again low for guillemots at Protection Island in 1984 compared to other data. Growth rates were:

Site	Year	# monit.	Ave. g/day	Reference
Hammersley In.	1984	8	13.43	this study
Protection Is.	1984	8	5.69	this study
Protection Is.	1979	35	12-14	Banks (1981)
Protection Is.	1980	31	12-14	Hirsch (1981)
Deception Pass	1957	42	12-14	Thorensen and Booth (1958
Mandarte Is.	1957-60	162	12-14	Drent et al. (1965)

The literature values for growth rates were approximated from age-weight tables and are not precise. These values, however, do indicate that the Protection Island growth rates were significantly lower than at Hammersley

Inlet (t-test, $p<.01$) and were lower than other values reported in the literature.

7.2.4 Eggshell thickness

Eggshell thicknesses of Pigeon Guillemots were not significantly thinner at target areas compared to reference areas and historical values. Forty-three whole eggs and eggshell fragments were obtained from our study sites (Table 31), 18 from Hammersley Inlet, 19 from Protection Island, and 6 from Seattle, Dumas Bay and Olympia combined. There was no significant variation in eggshell thickness between sites (ANOVA, $p>.05$). The eggshell thickness of the 18 Pigeon Guillemot eggs collected in Puget Sound by Oakley (1976) as a group were not significantly different from the thickness of eggs collected at any of our study sites (t-tests, $p>.05$). The eggshell thickness of eggs from each of our study sites and the Oakley study sites were lower but not significantly different from the thickness of eggs collected prior to 1947 in the Pacific Northwest (Henny et al., 1982)(t-tests, $p>.05$) .

7.2.5 Abnormalities

We found no external gross abnormalities in guillemot eggs and nestlings. No abnormalities were found in the shape or external appearance of the eggs observed at: Protection Island (n=38), Seattle (n=4), Tacoma-Dumas Bay (n=2), Olympia (n=4), and Hammersley Inlet (n=32). The nestlings handled at all sites appeared normal and without any obvious external abnormalities: Protection Island (n=11), Seattle (n=2), Olympia (n=1), and Hammersley Inlet (n=10).

7.2.6 Necropsy and histopathology

Results of gross and histological examinations of Pigeon Guillemots collected from Hammersley Inlet and Protection Island are summarized in Tables 32 and 33. The Pigeon Guillemots collected for this study had a much lower incidence of parasitism than the gulls. This difference probably reflects their relatively strict diet of fish from marine areas. The most consistent findings of pathology were ulcerations of the proventriculus and ventriculus consistent with eating sharp prey items. Only two birds (both from Protection Island) had tapeworms in the intestines. One bird from each site had intestinal flukes, and one bird from Protection had an infestation of Acanthocephalin worms. Livers of all guillemots were very similar in weight (average wt.= 28.4 g, range 23.5-33.7 g), with no birds exhibiting enlarged livers (50% greater than average) as with many of the gulls. There was no incidence of hepatitis and only one bird (from Protection Island) showed mild multifocal interstitial nephritis.

7.3 Conclusions

Populations of Pigeon Guillemots at Protection Island and Hammersley Inlet areas appear to be healthy while the status of this species at industrial waterfront areas is more difficult to determine. Reproductive success was low for guillemots in 1984 at Protection Island, our reference

Table 31. Eggshell thickness (with membrane) of whole eggs and egg fragments of Pigeon Guillemots, western Washington, 1984. No site values are significantly different from the mean pre-1947 value reported by Henny et al. (1982) for the "Pacific Northwest" (t-tests, $p>.05$).

Location	Number eggs measured	Mean thickness mm (s.d.)	Percent change, pre-1947	Reference
Hammersley Inlet	18	0.389 (.032)	0	This study
Protection Island	19	0.376 (.019)	-3	This study
Seattle, Olympia and Dumas Bay	6	0.379 (.032)	-2	This study
All sites (above)	43	0.382 (.027)	-2	This study
Puget Sound (1975)	18	0.386 (.019)	-1	Oakley (1975)
Pre-1947, Pacific Northwest	40	0.388 (.032)	---	Henny et al. (1982)

Table 32. Number of adult Pigeon Guillemots with specified gross lesions observed at necropsy.

Condition	Locations			
	Hammersley Inlet		Protection Island	
	No.	%	No.	%
No. examined	6	100	6	100
Tapeworm	0	0	2	33
Nematodes:				
Small	0	0	4	67
Large	0	0	1	17
Tetrameres	0	0	0	0
Crop paralysis	0	0	0	0
Gizzard:				
Erosions	2	33	3	50
Hemorrhage	0	0	0	0
Enlarged liver	0	0	0	0
Traumatic lesions	0	0	0	0
Bact. infects.	0	0	0	0
Spleen enlargement	0	0	0	0
Rt. oviduct*	0/4	0	0/1	0

* Enlarged or cystic right oviduct.

Table 33. Number of adult Pigeon Guillemots with specified histopathological conditions.

Condition	Locations: Hammersley Inlet No.	Hammersley Inlet %	Protection Island No.	Protection Island %
No. examined	6	6	6	6
Liver:				
Hepatitis	0	0	0	0
Fatty changes	0	0	0	0
Amyloidosis	0	0	0	0
Hepatocellular iron	1	17	1	17
Focal necrosis	0	0	0	0
Congestion	1	17	1	17
Heart:				
Mineralization	0	0	0	0
Myocarditis	0	0	0	0
Lung:				
Pneumoconiosis	0	0	1	17
Bronchitis	1	17	0	0
Tracheal nematodes	1	17	0	0
Esophagus:				
Ulceration	4	67	0	0
Lymphoid hyperplasia	0	0	0	0
Submucosal nematodes	0	0	0	0
Cysts	4	67	0	0
Proventriculus:				
Ulceration	2	33	3	50
Serosal granuloma	0	0	1	17
Proventriculitis	2	33	0	0
Intestine:				
Flukes	1	17	1	17
Mesenteric granuloma	0	0	0	0
Cloaca:				
Hemorrhage	0	0	0	0
Lymphoid hyperplasia	0	0	0	0
Flukes	0	0	0	0
Kidney:				
Multifoc. interstit. neph.	0	0	1	17
Amyloidosis	0	0	0	0
Flukes	0	0	0	0
Cystic tubules	0	0	0	0
Adrenals:				
Focal adrenalitis	0	0	0	0
Amyloidosis	0	0	0	0
Nervous system:				
Mineral deposits	2	33	0	0
Skeletal muscle:				
Sarcocysts	0	0	0	0
Hyaline fibers	0	0	0	0

area, but this population has been growing dramatically over the last two decades. Guillemots in Hammersley Inlet, a site of suspected moderate contaminant exposure, reproduced successfully and otherwise appeared healthy. There was only slight evidence for eggshell thinning occurring in guillemots and it did not occur to the significant degree found for gulls and herons. Though sample sizes were extremely limited, the hatching rate of guillemots in Seattle appeared low based upon a small sampling; only two of six eggs were seen to have hatched. Necropsy and histological examinations of guillemots revealed a relatively low incidence of parasitism or pathology.

8. CONCLUSIONS

A wide variety of biological parameters in marine mammals and marine birds were examined for evidence of a pattern that corresponded to the distribution of contaminants or matched documented effects of contaminants. Overall, populations of different species appeared to be doing fairly well. For almost all species examined, however, there was evidence of possible contaminant-related problems. This evidence included:

Seals at Gertrude Island, our primary target site in southern Puget Sound, had significantly higher incidences of skin lesions around the umbilicus and a higher incidence of a pelage anomaly than seals at other sites.

Killer whales that appear to be exposed to higher levels of contaminants have a lower reproductive rate, a higher mortality rate, and appear to be decreasing in numbers in recent years compared to killer whales suspected to be exposed to relatively lower levels of contaminants.

Harbor porpoise no longer occur in central and southern Puget Sound, areas of high contaminant levels where they used to be common.

Glaucous-winged Gull eggshells at all sites were significantly thinner than those from historical collections from the San Juan Islands with the greatest amount of thinning occurring at target sites.

Livers of Glaucous-winged Gulls were significantly heavier (enlarged) at Seattle compared to those from other areas. Liver weights of gulls at Tacoma, however, were the lowest.

The incidence of traumatic lesions (primarily injuries to feet and skin) in Glaucous-winged Gulls was significantly greater at target areas compared to reference areas.

Great Blue Heron eggshells were significantly thinner than those from historical collections at all study sites. The greatest amount of thinning occurred in herons from the northern bays near agricultural areas.

Of six Pigeon Guillemot eggs from Seattle, four did not hatch.

A number of conditions did not follow a geographical pattern consistent with the occurence of contaminants. Populations of harbor seals and Glaucous-winged Gulls appear to be increasing in all areas including target areas. Historical information for Pigeon Guillemot and Great Blue Heron populations at our target sites is limited but suggest these species are maintaining their numbers. Reproductive rates of all primary study species (harbor seals, Glaucous-winged Gulls, Great Blue Herons, and Pigeon Guillemots) appear to be within the range of normal values. River otter trapping records do not suggest any decline in otter in urban/industrial counties compared to other counties.

A cause and effect relationship between disorders and contaminants cannot be tested with the study design we employed. There is compelling information from other research that some of the disorders we observed, such as eggshell thinning are caused by contaminants. In other cases, such as with the incidence of traumatic lesions in gulls, environmental or behavioral factors unrelated to contaminants are the more likely cause.

Additional research will be required to further examine the possible relationship between contaminants and some of the disorders found in this study. Chemical analysis of tissues collected during this study would allow testing for correlations between some of the disorders described above and contaminant residue levels. Research specifically focusing on the problem areas described above would allow more thorough testing of the possible relationship between these disorders and contaminants.

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APPENDIX A: SUPPLEMENTAL TABLES

Table A-1. Reporting source of dead marine mammals recovered by species between 5 October 1983 and 29 January 1985. Fetuses are not included.

Species	Reporting source			
	Cascadia Research	MARC	Other stranding network	Cascadia contacts
Harbor seal				
Adults	4	6	4	2
Subadults	6	3	2	2
Pups	80	8	15	7
Harbor seal total	90	17	21	11
California sea lion *Zalophus californianus*	–	2	–	–
Northern sea lion *Eumatopias jubatus*	–	2	–	–
Northern fur seal *Callorhinus ursinus*	–	–	1	–
Harbor porpoise *Phocoena phocoena*	–	1	–	–
Killer whale *Orcinus orca*	–	1	–	–
Minke whale *Balaenoptera acutorostrata*	–	–	1	–
Gray whale *Eschrichtius robustus*	–	3	–	–
River otter *Lutra canadensis*	–	–	1	22
Mink *Mustela vision*	–	–	–	7
Total	90	26	24	40

Table A-2. Description of areas searched at harbor seal haul-out sites. Contacts made for additional search coverage are listed.

Site	Description	Other coverage
Southern Puget Sound		
Gertrude Island	- perimeter of Gertrude Is. and E and W shore of Still Harbor on McNeil Is. by foot - W shore of Still Harbor and NE McNeil Is. by boat - N McNeil Is. between Still Harbor and Wyckoff Shoal*	
Henderson Inlet	- all logs and booms at the Woodard Bay log dump and small boom just N of the log dump by boat and foot	workers at the log dump
Budd Inlet	- all logs and boom maintained by the Dunlop Towing Co. by boat and foot	workers at the log dump
Eld Inlet	- all recreational floats in the inlet by boat	Eld Inlet residents
McMicken Island	- NE shore on foot	caretaker who lives on the island
North of Puget Sound		
Smith Island	- E Smith Is., all Minor Is., and the connecting spit by foot - perimeter of Smith Is. on foot*	
Protection Island	- the N and E shore of Violet Point and the S and W shores of Kanem Pt. by foot - perimeter of Protection Is. by boat*	W. Reed, U.Wash. & USFWS personnel
Skipjack Island	- reef haul-out site during low tide by foot - perimeter of the island and in kelp beds by boat	
Hood Canal		
Skokomish Delta	- shore of Skokomish River and all sloughs E and W of the delta	
Duckabush Delta	- shore of Duckabush River and all sloughs N and S of the delta	residents near delta
Dosewallips Delta	- shore of Dosewallips River and all sloughs N of the delta	Wa. Dept. Parks

* - areas checked less frequently than the other areas

Table A-3. Samples collected from marine mammals for histopathology and future contaminant analysis, from 5 October 1983 to 29 January 1985.

Species	# animals examined	# samples collected: Chlor hydro. Bl	Mu	Li	Br	Heavy metals Li	Ki	Sp	Histo path
Harbor seal *Phoca vitulina*	142	112	91	95	55	94	97	87	74
California sea lion *Zalophus californianus*	2	2	1	2	-	2	2	-	2
Northern sea lion *Eumatopias jubatus*	2	2	-	1	-	1	1	1	1
Northern fur seal *Callorhinus ursinus*	1	1	1	1	-	1	1	1	1
Killer whale *Orcinus orca*	1	1	1	1	-	1	1	1	1
Minke whale *Balaenoptera acutorostrata*	1	1	1	1	-	1	1	-	-
Gray whale *Eschrichtius robustus*	3	3	1	1	1	1	1	-	-
River otter *Lutra canadensis*	23	23	22	23	-	23	23	20	-

Codes: Bl=blubber, Mu=muscle, Li=liver, Br=brain, Ki=kidney, Sp=spleen.

Table A-4. Samples collected for histopathology, microbiology, and future contaminant analysis from harbor seals by region and age/sex class, 5 October 1983 to 29 January 1985. Table does not include the one harbor seal pup from Grays Harbor.

Region & Age class	# Exam.	# Animals Sampled: Chlor. Hydro.	Heavy Metal	Hist.	Bact.	Vir.	Lepto. Pup	Prot.	Repro. Tract.
Southern Puget Sound (n=62)									
Adult M	2	2	2	0	0	0	-	0	-
Adult F	4	4	4	4	0	0	-	3	3
Adult U	2	0	0	0	0	0	-	0	-
Subadult	10	6	5	3	2	1	-	2	-
Pup	43	42	38	27	23	23	8	19	-
Fetus	1	1	1	1	1	0	0	0	-
SPS total	62	55	50	35	24	24	8	24	3
Hood Canal (n=26)									
Adult M	0								
Adult F	5	5	5	4	2	2	-	1	1
Adult U	2	0	0	0	0	0	-	0	-
Subadult	1	1	1	1	1	1	-	1	-
Pup	16	7	5	3	3	4	1	2	-
Fetus	2	2	2	1	1	1	0	1	-
HC total	26	15	13	9	7	8	1	5	1
North of Puget Sound (n=53)									
Adult M	1	1	1	1	1	0	-	0	-
Adult F	0								
Adult U	0								
Subadult	2	1	0	0	0	0	-	0	-
Pup	50	39	35	29	17	16	9	16	-
Fetus	0	—	—	—	—	—	—	—	—
NPS total	53	41	36	30	18	16	9	16	0
Total	141	112	100	74	49	48	18	45	4

Codes: # Exam.= # animals examined, Chlor. Hydro= Chlorinated Hydrocarbons, Hist.= Histopathology, Bact.= Bacteriology, Vir.= Virology, Lepto. Pup= Leptospirosis of pups, Prot.= Protein analysis, Repro. Tract.= Reproductive tracts of adult females.

Table A-5. Tissue samples collected from complete necropsies of marine mammals.

CHEMICAL ANALYSES frozen and stored by Cascadia Research in Olympia, Wa.

Chlorinated hydrocarbons:
(in super cleaned jars)
- Blubber
- Muscle
- Liver
- Brain
- Stomach or esophogus contents

Heavy metals:
(in whirl pacs)
- Kidney
- Liver
- Spleen

BACTERIOLOGY sent to Oregon State University

Leptospirosis:
(in lrg vials w/ medium, stored room temp)
- Liver
- Kidney
- Urine
- Placenta

Viral analysis:
(break dacron swab off into cold sm. vial w/ medium,refrigerate)
- Nose
- Throat
- Anus

Bacterial Identification sent to Oregon State Univ.
(culturette swab)
- Brain
- Liver
- Respiratory tract

PATHOLOGY sent to Evergreen Professional Service (Veterinary pathologist), Kirkland, Wa.

Histopathological
(in 10% buffered formalin)
- Salivary glands
- Thyroid
- Lymph node(sal.gland)
- Lymph node(pectoral)
- Heart
- Thymus
- Lung
- Rib
- Liver
- Pancreas
- Spleen
- Lymph node(spleen)
- Adrenals
- Kidney
- Stomach
- Colon
- Umbilicous junction (if pup)
- Ovaries or repro. tract
- Testes
- Skin
- Muscle
- Blubber
- Brain tissue
- Pituitary gland
- Placenta
- Any lesions

PROTEIN ANALYSIS:to Debbie Duffield Portland State Univ.
- Blood (red top tubes)
- Eye

OTHER:
- Skull
- Canines
- Lower jaw
- Stomach+contents

Table A-6. Census results for harbor seal haul-out areas in southern and central Puget Sound in 1984.

Site	Obs. dates		Land	Obs.	Air	Peak counts			High count		Max. pups	
	Start	end	vis.	hr	surv.	n	mean	sd	#	date	#	date
Eld Inlet	4 /9	9 /28	46	46	8	18	28	12	53	9 /9	7	8 /3
Budd Inlet	1 /2	11/6	57	81	5	36	27	14	59	9 /15	8	9 /12
Henderson	1 /22	12/30	55	130	8	29	120	55	228	9 /16	52	8 /7
McMicken Is.	3 /6	9 /9	5	11	6	10	18	18	50	5 /12	0	8 /7
Gertrude Is.	4 /27	11/21	73	359	9	59	324	116	483	9 /24	77	10/5
Nisqually	4 /27	9 /9	0	0	5	2	11	8	28	8 /7	3	8 /7
Oakland Bay	4 /27	9 /9	0	0	6	6	5	6	16	8 /7	1	8 /7
Totten Inlet	6 /9	9 /9	0	0	4	4	2	3	6	9 /9	0	8 /7
Wyckoff Shoals	4 /27	9 /9	0	0	5	5	43	27	82	6 /9	1	8 /7
Eagle Is.	4 /27	9 /24	0	0	6	6	16	7	25	7 /11	2	9 /9
Cutts Is.	6 /9	9 /9	0	0	3	1	28	0	28	9 /9	0	9 /9
Poulsbo	8 /24	8 /24	0	0	1	0	0	0	11	8 /24	2	8 /24
Other Carr In.	6 /9	9 /9	0	0	3	1	10	0	10	6 /9	0	9 /9
Other Case In.	7 /11	8 /7	0	0	2	1	6	0	6	7 /11	2	8 /7

Land vis.--Number of visits by land to count seals
Obs. hr.--Hours spent counting seals
Air Surv.--Number of aerial surveys flown over site to count seals
Peak counts--Number of counts, mean of the high counts, and standard deviation for counts made when peak numbers of seals were expected

Table A-7. Census results for harbor seal haul-out areas in Hood Canal in 1984.

Site	Obs. dates Start	Obs. dates end	Land vis.	Obs. hr	Air surv.	Peak counts n	Peak counts mean	Peak counts sd	High count #	High count date	High pup #	High pup # date
Skokomish	1 /15	11/7	53	160	7	47	165	89	428	10/26	29	9 /19
Hamma Hamma	5 /30	10/26	24	11	5	9	76	37	131	10/5	14	9 /13
Duckabush	2 /11	10/26	42	70	4	27	144	96	302	8 /30	33	8 /30
Dosewallips	2 /11	10/26	41	59	4	30	166	90	339	9 /13	27	9 /13
Quilcene	6 /17	10/12	3	1	4	4	117	51	155	7 /15	12	9 /13
Port Gamble	5 /14	8 /24	0	0	4	0	0	0	5	7 /13	0	9 /13

Land vis.--Number of visits by land to count seals
Obs. hr.--Hours spent counting seals
Air Surv.--Number of aerial surveys flown over site to count seals
Peak counts--Number of counts, mean of the high counts, and standard deviation for counts made when peak numbers of seals were expected

Table A-8. Census results for harbor seal haul-out areas north of Puget Sound in 1984.

Site	Obs. dates Start	end	Land vis.	Obs. hr	Air surv.	Peak counts n	mean	sd	High count #	date	High pup #	date
Smith & Minor	5 /3	9 /13	48	420	7	29	181	107	546	9 /13	75	7 /16
Violet Pt., PI	5 /3	10/3	30	30	6	1	175	0	320	9 /13	50	8 /8
Kanem Pt., PI	5 /3	9 /13	16	14	6	5	65	17	91	7 /13	5	8 /12
San Juan Is.*	5 /14	8 /24	0	0	4	4	1553	217	1800	8 /24	147	8 /24
Skipjack Is.**	5 /5	8 /13	6	19	4	8	143	36	216	6 /11	16	7 /16
NW of Sequim	5 /14	8 /24	0	0	4	4	11	11	26	7 /13	0	8 /12
Dungeness	5 /14	9 /13	0	0	5	4	246	147	404	6 /11	37	8 /24
Marrowstone Is.	5 /14	8 /24	0	0	4	3	37	29	70	8 /24	7	8 /24
Klaus Rocks	7 /13	8 /24	0	0	3	3	15	15	31	8 /24	4	8 /24
Belling. Bay	5 /14	7 /13	0	0	4	2	1	1	2	7 /13	0	8 /24
Samish Bay	5 /14	8 /24	0	0	4	3	26	44	77	8 /24	6	8 /24
Padilla Bay	5 /14	8 /24	0	0	4	3	149	19	168	7 /13	23	8 /24
Eliza Is.	5 /14	8 /24	0	0	4	4	26	21	47	8 /24	3	8 /24
Hat Is.	5 /14	6 /11	0	0	2	1	0	0	0	8 /24	0	8 /24
Simil Bay	8 /24	8 /24	0	0	1	0	0	0	12	8 /24	0	8 /24

* San Juan's include numerous haul-out areas throughout U.S. San Juan Is.
** Includes adjacent Bare Is., aerial counts also included in San Juan counts
Land vis.--Number of visits by land to count seals
Obs. hr.--Hours spent counting seals
Air Surv.--Number of aerial surveys flown over site to count seals
Peak counts--Number of counts, mean of the high counts, and standard deviation for counts made when peak numbers of seals were expected

Table A-9. Marine mammal census results for 1984 aerial surveys where most seal haul-out areas in an entire region were covered. Counts are separated into those at primary haul-out sites (in Table 5 plus Protection Is.) and those at other sites. Counts after a / refer to pup counts.

Date	No. harbor seals			Other species	Comments
	Prim.	Other	Total		
Southern Puget Sound					
27 April	162	48	210	64 Zc 102 Ej	
9 June	163	129	292	3 Ej 1 Er	
11 July	295/3	143	438/3		Not incl. Case Inlet
7 Aug.	248/37	132/8	380/45		
9 Sept.	624/64	95/2	719/66		Two flights
Hood Canal					
17 June	486	235	721		
15 July	393	175	568		
29 Aug.	675/55	124/17	799/72		Quilcene seals gone
13 Sept.	629/58	192/19	821/77		No Skok. count
North of Puget Sound					
14 May	1497	150	1647	31 Zc 2 Er	Post-peak in N bays
11 June	1971/4	564	2535/4		
13 July	2155/112	509/1	2664/113	1 Er	
24 Aug.	2393/190	679/80	3072/270	1 Pd	

Zc-California sea lion, Ej-northern sea lion, Er-gray whale, Pd-Dalls porpoise

Table A-10. Marine mammal census results for 1984 aerial surveys involving only specific sites within a region. Counts are separated into those at primary haul-out sites (in Table 5 plus Protection Island) and those at other sites. Counts after a / refer to pup counts.

Date	No. harbor seals			Other species	Comments
	Prim.	Other	Total		
Southern Puget Sound					
14 May	223	0	223	6 Zc 15 Ej	McNeil area only
29 Aug.	15	10	25		Eld and Shelton only
14 Sept.	49	0	49		Eld only
24 Sept.	498/39	11	509/39		Gertrude, Henderson, and Eagle Is. only
28 Sept.	48	0	48		Eld Inlet only
29 Oct.	525	0	525	1 Ej	Gertrude & Hend. only
14 Nov.	263	0	263	1 Zc 3 Ej	Gertrude area only
Hood Canal					
2 July	84	95	179	3 Pd	Skok. & Hamma H. only
14 Sept.	133/7	0	133/7		Skok. only
28 Sept.	257/14	0	257/14		Skok. only
15 Nov.	40	2/1	42/1		Skok.-Belfair only
North of Puget Sound					
17 June	427/13	0	427/13		Smith only
15 July	343/51	1	344/51	2 Er 3 Pd	Smith and Protec. only
13 Sept.	891/19	143/4	1034/23		Smith, Protec., and Dungeness only

Zc-California sea lion, Ej-northern sea lion, Er-gray whale, Pd-Dalls porpoise

Key to Appendix Table A-11 and A-12:

CD (cause of death, Pri-primary cause, Sec-secondary cause):
- 0-Not determined
- 1-Emaciation
- 2-Stillborn
- 3-Drowning
- 4-Coyote kill
- 5-Shot
- 6-Other trauma
- 7-Died or euthanized in captivity
- 8-Blow to head
- 9-Dead fetus
- 10-Drown in gillnet
- 11-Premature
- 12-Emaciation after drowning
- 13-Septicemia
- 14-Other suspected infectious agent
- 15-Myocardial necrosis
- 16-Pneumonia

Age:
- P-pup
- PR-premature
- F-fetus
- Pl-placenta

Blub=blubber thickness

Microbiology:
- NSI- no significant isolates
- PC- pure culture
- Acinetob.- Acinetobacter sp.
- a-strep- Alpha-streptococcus sp.
- b-strep- Beta-streptococcus sp.
- Colif- coliforms
- Coryn- Corynebacterium sp.
- Ec- Escherichia coli
- Enterob.- Enterobacter sp.
- Influ.- influenza virus
- Past- Pasteurella sp.
- Pseudom.- Pseudomonas sp.
- Reo- reovirus

Histology
- Atr.- atrophy
- Pigm.- pigment
- Umbil.- umbilicous
- Aspir.- aspirated
- Hepat.- hepatic
- Miner.-mineralization
- Enl.-enlarged
- Necr.-necrosis
- Congest.-congestion
- Mecom.- meconium
- Infl.- inflammation

Other abbreviations:
- pm- post-mortum
- Nod.- node
- Emac.-emaciation
- Umb.-umbilicous
- Abdom.- abdominal
- Cav.- cavity

Table A-11. Summary information on all harbor seal pups found dead from 5 October 1983 to 8 January 1985, and information on live pups and placentas sampled included only when they were sampled for microbiology. See key for code explanations.

Sam. #	Date mo	dy	yr	Age	Sex	Location	Len cm	Girth cm	Wt kg	Blub cm	CD Pri	CD Cont	Gross findings	Micro.	Histology
22	10	22	83	P	F	Anacortes	93	62	18.0	1.7	5	-	Shot in back		
25	11	23	83	P	M	Purdy	85	50	9.5	1.3	0	-	Blood in pleural cav.		
50	5	4	84	PR	M	Smith	61	32	4.5	0.5	11	-	Blood in pleural and abdom. cav.		No sign. pathology
51	5	4	84	PR	F	Smith	66	25	-	-	11	2	Stillborn		
58	5	16	84	PR	U	Smith	-	-	-	-	11	-	Lanugo coat and skin only		
57	5	18	84	PR	M	Smith	72	36	6.0	1.0	11	2	Stillborn, blood in abdom.		No sign. pathology
60	5	18	84	P	F	Grays Harbor	79	47	9.4	1.2	2	-			
61	5	21	84	PR	M	Gertrude	57	34	3.9	0.5	11	2	Stillborn		Not determined
63	5	28	84	PR	U	Protection	61	-	-	-	11	-			
64	5	28	84	PR	U	Protection	63	-	-	-	11	-			
73	6	1	84	PR	M	Smith	74	38	7.1	1.0	11	-		Ec	Mild interstitial pneum. thymic atr,liver atr & pigm.
74	6	2	84	PR	M	Smith	78	37	6.5	0.6	11	2	Stillborn	Ec	Same as above
75	6	2	84	PR	F	Smith	76	38	6.5	0.5	11	14	Poss. liver lesions	Ec	Same as above
76	6	2	84	PR	U	Smith	-	-	-	-	11	-	Skin and backbone only		
77	6	5	84	Plac.		Smith					-	-	Placenta only	b-strep.	
80	6	13	84	PR	M	Smith	78	43	9.0	1.4	11	-	Placenta attached, scoliosis		Hepatic atr & pigm., mild thymic atr.
81	6	13	84	P	M	Smith	87	43	10.5	1.1	0	-	Umbilicus, poss. liver lesions		
87	5	29	84	PR	U	Protection	60	-	-	-	11	-	Length approx.		Equivocal thymic atr.
88	6	22	84	P	M	Smith	89	50	11.8	1.1	2	-	Umbilicus, blood in abdom., liver rupt.	Influ.	No sign. pathology
91	6	26	84	PR	M	Smith	72	52	4.8	1.7	11	-			
92	6	20	84	PR	M	Henderson	74	-	7.3	-	7	-	w/ lanugo, recovered live then died		
94	6	30	84	P	F	Smith	79	46	9.5	1.0	0	-	Umbil.,large lymph no.,pos. eagle pred.	NSI	Hepatic atr. & pigm.
95	7	1	84	P	M	Smith	80	37	7.1	0.5	14	1	Umbilicus, milk in stomach	Reo virus	Mild interstitial pneum., lymphoid hyperplasia, thymic at
96	7	3	84	P	M	Smith	78	46	8.6	1.1	0	13	Umbilicus, blood in abdom., poss. liver abnor.	Influ.,b-strep.	Umbil. infec., thymic atr., septicemia
97	7	6	84	P-L	M	Smith	76		9.		-	-	Live pup, eye infect.,blind,emac.,umb.	Influ., Coryn.-eye	

Table A-11 (cont.). Summary information on pups found dead.

Sam. #	Date mo dy yr	Age	Sex	Location	Len cm	Girth cm	Wt kg	Blub cm	CD Pri	CD Cont	Gross findings	Micro.	Histology
100	7 11 84	P	F	Eld	83	54	12.7	1.5	0	-	Placenta attached, froth in lungs	Past. hemolytica Influ.	No sign. pathology
101	7 11 84	P	F	Smith	87	51	11.4	1.5	8	-	Blood in abdom., trauma to head		
102	7 11 84	P	M	Smith	86	50	11.4	1.4	2	-	Umbilicus		
103	7 11 84	PR	F	Protection	71	40	-	-	11	-			
104	7 11 84	PR	M	Smith	74	41	6.0	0.7	11	-			
105	7 15 84	P	M	Smith	74	35	5.4	0.3	1	-	Froth in lungs, obser. starving 7 days	Ec, Proteus	Mild hepatic atr.
106	7 13 84	P	F	Smith	76	43	7.2	0.5	1	13	Froth in lungs, blood in abdom., poss liver abnorm., infect. umbil.	Ec, Proteus	Umbil. inf., septicemia hepatic & thymic atr.
107	7 13 84	P	M	Smith	87	46	10.5	1.2	0	-	Poss liver lesions, froth in lungs	Ec, Proteus	Aspir. mec., hepatic pigm.
108	7 14 84	P	M	Smith	81	39	8.3	0.6	1	-	Blood in abdom., froth in lungs		Not determined
109	7 14 84	P	F	Smith	72	37	5.8	0.8	1	13	Umbilicus, blood in abdom., bleeding from umb. while alive	Ec, Proteus	Umbil inflam., septicemia,
110	7 11 84	P	M	Smith	82	52	9.0	0.5	1	-			
111	7 14 84	P	F	Henderson	89	57	14.1	1.6	2	14	Umbilicus, blood in abdom., froth in lungs, died at birth site	NSI	Thymic and hepat. atr., bile plugs, prob. fetal infec.
112	7 14 84	P	F	Smith	77	40	7.3	0.5	1	-	Froth in lungs		
113	7 17 84	P	F	Smith	81	44	7.8	0.4	1	-	Froth in lungs	NSI	Lymphoid hyperplasia, hep. pigm. & lipid
114	7 17 84	P	M	Smith	83	43	8.3	0.7	2	-	Froth in lungs, thymus small		
115	7 17 84	P	F	Protection	84	-	8.5	-	0	-			Aspir. amniotic fluid
118	7 16 84	F	M	Budd	90	63	13.2	3.0	9	-	Placenta attached, crushed skull prob pm	Colif.	Myocardial necrosis and
119	7 23 84	P	F	Protection	85	51	10.5	1.2	15	2			miner., thymic necrosis
120	7 22 84	P	M	Waldron Is.	68	-	-	-	7	-	necr by Dr.Jessica Porter		
121	7 24 84	P	M	Smith	83	48	6.9	1.1	2	-	Prob stillbirth		
122	7 24 84	P	M	Smith	81	49	8.9	0.9	0	-	Blood in pleural cavity	Ec, Proteus	Pulmonary edema
123	7 24 84	P	M	Smith	80	48	6.9	0.5	1	-	Fractured skull prob pm		Pulmonary edema
124	7 24 84	F	M	Squamish Hbr	62	31	2.3	0.3	9	-	Fetus of CRC-124, skull fract.		
125	7 29 84	P	M	Henderson	88	71	9.3	2.5	0	-			
126	7 30 84	P	M	Budd	89	64	11.4	1.5	0	-	Umbilicus, fractured skull and ribs, trauma to head		
127	7 30 84	P	M	Budd	84	60	8.7	1.1	0	-	Umbilicus, blood in pleural cav., skull fract poss pm		

Table A-11 (cont.). Summary information on pups found dead.

Sam. #	Date mo	dy	yr	Age	Sex	Location	Len cm	Girth cm	Wt kg	Blub cm	CD Pri	CD Cont	Gross findings	Micro.	Histology
130	6	25	84	P	U	Skipjack Is.	80	-	-	0.5	0	-			
131	7	23	84	P	M	Henderson	87	61	10.5	1.4	2	-	Suspected stillbirth	Colif.	Thymic atr.
132	7	23	84	P	M	Henderson	82	50	9.1	1.0	0	-			
133	7	23	84	P	F	Henderson	83	64	10.5	2.0	0	-	Possible stillbirth		
135	7	22	84	P	M	Bellingham	82	38	7.3	0.2	13	-	MARC live, dead to us, froth in lungs	Ec, Proteus	Umbil. infec., septicemia, hepatitus, thymic atr.
137	8	4	84	P	F	Henderson	81	43	9.1	1.4	2	-	Skull fractured prob pm		
138	8	8	84	P	M	Smith	88	51	9.0	1.6	0	-	Est. total wt		
139	8	8	84	P	M	Smith	81	43	8.3	0.5	1	13	Umbilicus,poss liver lesions, froth in lungs	Ec	Umbil. inflam, septicemia, thymic atr.,hep. atr. & pigm.
140	8	8	84	P	F	Protection	79	46	6.3	0.8	0	-	Skull fractured prob pm		
141	8	8	84	P	F	Protection	90	47	8.0	1.5	0	-	Wt and girth estimated		
142	8	8	84	PR	U	Protection	-	-	-	-	11	-			
143	8	6	84	PR	U	Protection	85	-	-	-	11	-	Length estimated		
144	8	7	84	P	F	Henderson	80	65	9.5	1.7	0	-	Length estimated		
146	8	8	84	P	M	Duckabush	82	-	-	-	0	-	Length estimated		
147	8	11	84	P	M	Duckabush	86	42	9.1	1.3	2	-	Lungs partially aerated	Colif.,Proteus	Pulmonary edema
148	8	17	84	P	U	Skokomish	80	-	-	-	0	-	Length estimated		
149	8	17	84	P	U	Skokomish	85	-	-	-	0	-	Length estimated		
150	8	20	84	P	M	Boston Harb	90	69	23.5	4.0	10	-	Pup of the year, froth in lungs	NSI	Severe edema in lungs
151	8	20	84	P	M	Danas Pass	92	62	21.0	4.0	3	-	Pup of the year, froth in lungs,	b-strep.	Lungs congest. & edema
152	8	21	84	PR	F	Smith	59	-	3.3	-	11	-			
153	8	24	84	P	F	Danas Pass	84	62	17.0	3.0	10	-	Pup of the year, froth in lungs		
154	8	22	84	P	F	Sequim Bay	83	46	8.7	-	0	-			
155	8	25	84	P	F	Boston Harb	99	69	23.5	3.0	10	-	Pup of the year, froth in lungs,	NSI	Lungs congest. & edema
156	8	26	84	P	U	Duckabush	-	-	-	-	0	-			
157	8	20	84	P	M	Gertrude	82	53	10.0	1.2	0	-	Poss. coyote kill		Not suitable
158	8	21	84	P	F	Gertrude	88	47	10.0	1.4	13	-	Froth in lungs	a-strep.	Septicemia, mod. thymic atr., lymphoid necr.
159	8	22	84	P	F	Gertrude	70	38	8.5	0.8	4	13	Trauma to head and abdom. cav.	NSI	Umbil. infec., septicemia

Table A-11 (cont.). Summary information on pups found dead.

Sam. #	Date mo	dy	yr	Age	Sex	Location	Len cm	Girth cm	Wt kg	Blub cm	CD Pri	CD Cont	Gross findings	Micro.	Histology
160	8	22	84	P	M	Gertrude	82	44	10.0	0.8	0	14	Froth in lungs, length est.	NSI	Marked thymic atr., lymph nodes enl., suspected perinatal death from septicemia
161	8	26	84	P	M	Duckabush	85	53	4.2	2.5	0	-	Weight is min.		
162	8	29	84	PR	U	Protection	-	-	-	-	11	-			
164	8	30	84	P	M	Dosewallips	88	45	11.0	1.0	2	-	Umbilicus present, weight est.		
165	8	30	84	P	M	Dosewallips	94	47	13.0	1.3	2	-	Froth in lungs	Proteus(PC)	Thymic atr., congest. & edema in lungs
166	8	30	84	P	M	Gertrude	80	47	10.0	1.2	4	14	Froth in lungs,trauma to head, thymus small	Colif.	Severe thymic atr., lymphoid hyperplasia,
167	8	30	84	P	M	Gertrude	93	55	15.9	1.7	4	13	Trauma to head	Colif.	Umbil. infl., septicemia, thymic atr., infl. in lymph nodes
168	8	31	84	P	F	Skokomish	86	54	16.0	2.0	0	-		Colif.	Lung cong. & edema
169	8	31	84	P	F	Skokomish	86	-	6.8	0.6	1	-	Prob. emaciation		
170	9	2	84	P	M	Gertrude	77	52	10.9	1.5	0	-	Umbilicus, froth in lungs	Colif.	Equiv. thymic atr., lungs cong. & aspir. mecom.
173	9	9	84	P	F	Gertrude	81	38	7.3	0.4	1	-	Froth in lungs	NSI	Not suitable
174	9	4	84	P	F	Boston Harb	87	63	18.5	3.0	10	-	Pup of the year, froth in lungs		Hepatic pigm., cong. & edema in lungs
175	9	11	84	P	F	Whidbey Is.	91	63	16.0	3.0	8	3	Pup of the year, froth in lungs, trauma to head	Proteus	Thymic atr., congest. & edema in lungs
176	9	7	84	P	F	Seattle	90	48	9.2	0.2	12	13	Pup of the year, froth in lungs, enlarged lymph nodes	NSI	Cellulitus, congest. & edema in lungs
179	9	15	84	P	M	Rosedale	91	44	10.4	0.3	1	14	Froth in lungs, enlarg. lymph nodes	b-strep(PC)	Thymic atr., aspir. mecom., lymphoid hypertrophy, prob. fetal illness
180	9	14	84	P		Skokomish	86	-	-	-	0	-			
181	9	14	84	P	M	Skokomish	79	-	10.0	-	1	-	Marked while live		
182	9	15	84	P	M	Gertrude	88	49	11.8	1.2	8	3	Froth in lungs, trauma to head, enlar. lymph nodes, milk in stom.	Pseudom.	Lungs congest., thymic atr.

Table A-11 (cont.). Summary information on pups found dead.

Sam. #	Date mo	dy	yr	Age	Sex	Location	Len cm	Girth cm	Wt kg	Blub cm	CD Pri	CD Cont	Gross findings	Micro.	Histology
183	9	18	84	F	F	Twanoh	85	52	13.6	1.5	9	14	Fetus of CRC-185, bullous emphysema	Proteus	Mod. thymic atr., lungs cong. w/ mecom. & squames prob. fetal illness
184	9	18	84	P		Hope Is.	99	74.	20.0	3.6	8	3	Froth in lungs, blood in abdom. cav., trauma to head		
186	9	17	84	P	F	Gertrude	91	52	12.0	1.6	4	-	Congealed blood outside of kidney, trauma to body		
187	9	25	84	P	U	Skokomish	-	-	-	-	0	-			
188	10	2	84	PR	M	Skokomish	80	-	-	-	11	-			
189	10	4	84	P	F	Gertrude	90	47	13.6	1.3	4	-	Froth in lungs, coyote attack obs., trauma to head and body		Mild thymic atr.
190	10	7	84	P	F	Rosedale	92	58	15.9	2.9	8	3	Froth in lungs, enlar. lymph nodes, trauma to head	Enterob.	Mild thymic atr., severe congest & edema in lungs
193	10	8	84	P	F	Gertrude	85	43	10.0	1.1	13	-	Umbilicus, blood in abdom. cav.	NSI	Umbilic. inflam., septicemia, infl. lymph node reduced colloid in thyroid
194	9	26	84	P	F	Keyport	84	44	7.5	0.3	1	-	Froth in lungs, pocket of blood near liver		
195	10	14	84	P	F	Twanoh	91	67	13.5	1.5	8	-	Umbilicus, trauma to head		
197	10	20	84	P	U	Gertrude	-	-	-	-	4	-	Trauma to head, only upper torso		
198	10	26	84	P	U	Skokomish	-	-	-	-	0				
200	10	27	84	P	M	Gertrude	-	-	-	2.8	4	-	Bottom half only		
202	11	11	84	P	F	Purdy	84	44	8.0	0.2	12	-	Blood in abdom. cav. poss. pm		
203	11	11	84	P	F	Purdy	80	52	7.3	0.5	12	-	Blood in abdom. cav. poss. pm		
205	8	29	84	P	M	Boston Harb	95	-	20.0	3.5	10	-	Pup of the year, froth in lungs, trauma to head, wt. est.		
209	11	8	84	P	F	Purdy	93	64	19.0	2.5	5	-	Pup of the year, trauma to head		
211	12	19	84	P	M	Elliot Bay	96	55	-	1.0	3	-	Pup of the year, froth in lungs		
214	1	8	85	P	F	Steilacoom	89	54	11.8	0.5	7	16	Pup of the yr,wounded,emac.,euth., blood in abdom cav.,lung worm, adren.lrg	Proteus	Pneumonia

Table A-12. Summary information on all harbor seal adults and subadults found dead from 5 Oct. 1983 to 8 Jan. 1985. See key for code.

Sam. #	Date mo	dy	yr	Age	Sex	Location	Len cm	Girth cm	Wt kg	Blub cm	CD Prim	CD Cont.	Gross findings	Microbiology	Histology
21	10	5	83	A	M	Steilacoom	163	117	100.0	2.0	6		Broken ribs, poss. pm		
24	11	16	83	A	F	Belfair	157	102	83.5	2.7	8		Trauma to head, blood in abdom.		
32	1	11	84	S	U	Purdy	117	-	-	>1.9	0				
33	1	11	84	S	F	Hope Is.	118	-	-	3.5	0				
34	2	4	84	A	M	Purdy	165	117	86.0	2.0	8		Trauma to head, blood in pleural cav.		
48	4	9	84	S	M	Eld Inlet	142	91	52.0	1.7	8		Blood in cav, poss pm		
56	5	16	84	S	F	Smith	127	98	82.0	-	0		Poss. skeletal abnorm.		
59	5	17	84	A	U	Gertrude	-	-	-	-	0		Skeleton only		
62	5	21	84	S	U	Gertrude	-	-	-	-	0		Skeleton only		
78	6	6	84	S	M	Devils Head	93	56	15.0	0.8	3	1	Poss. liver lesions		
79	8	6	84	A	U	Duckabush	-	-	-	-	0		Skull only		
89	6	23	84	S	U	Gertrude	-	-	-	-	0		Skull only		
117	7	16	84	A	F	Budd	145	110	90.0	2.0	0		w/fetus		Skin ulcer and inflam.
124	7	24	84	A	F	Sqaumish Hbr	160	107	68.0	3.0	0		w/fetus, fract skull prob pm		
134	7	25	84	A	F	Budd	160	119	90.0	3.1	0		Blood in pleural cavity, post-partum		
145	8	8	84	A	U	Gertrude	-	-	-	-	0		Lower jaw only found		
171	9	1	84	A	F	Seal Rock	153	116	90.0	5.1	8		Froth in lungs, post-partum, trauma to head	Colif.	NS
172	9	9	84	S	U	Gertrude	-	-	-	-	0		Skull only		
185	9	18	84	A	F	Twanoh	159	108	100.0	3.7	8		w/fetus, blood in abdom., trauma to head, large lymph nod.		
191	10	9	84	S	M	Vashon Is	105	69	22.0	1.5	3			Proteus	Severe cong. & edema lungs
192	5	4	84	S	U	Smith	-	-	-	-	0		Skull only		
196	10	18	84	S	M	Seabeck	101	64	22.5	2.3	5		Froth in lungs, trauma to head		
199	10	27	84	S	U	N McNeil	-	-	-	-	0		Skull only		
201	10	31	84	S	M	Nisqually	105	67	25.5	2.3	10		Froth in lungs, poss. liver lesions		
204	4	6	84	A	U	Skokomish	-	-	-	-	0		Skull only		
206	11	21	84	A	F	Longbranch	144	89	59.1	2.4	5		Post partum, blood abdom., large lymph no., poss. repro abn.		
210	12	11	84	A	F	Horsehead By	149	97	57.0	2.5	0		Poss. liver lesion,large lymph no.,poss. early preg.,trauma to head		
212	12	27	84	A	F	near Twanoh	145	84	55.0	2.4	8		Trauma to head		
213	1	7	85	A	M	Whidbey Is	161	86	63.0	1.3	14	1	Emaciated,growth in lungs, yellow fluid in resp. & dig. tracts	Acinetob.	Severe pneumonia

Table A-13. Killer whale gross recruitment and mortality rates by region and pod type (resident or transient). Letters under pod-type refer to individual pods.

	S Resident Pods(1)				N Resident Pods(2)				Transient Pods(2)
	J	K	L	Total	A5	A1	A4	Total	Total
1973-1980									
Cow years	64	35	154	253	37	35	24	96	NA
Pod years	117	70	341	528	67	99	34	200	NA
Births	5	1	11	17	4	3	2	9	NA
Deaths	1	1	1	3	2	3	0	5	NA
Birthrate/ cowyear(%)	7.8	2.9	7.1	6.7	10.8	8.6	8.3	9.4	
Mortality/ pod-year(%)	.85	1.4	.29	.57	3.0	3.0	0.0	2.5	
1981-1984									
Cow years	31	20	81	132	NA	NA	NA	NA	NA
Pod years	73	40	192	305	NA	NA	NA	NA	NA
Births	2	0	4	6	NA	NA	NA	NA	NA
Deaths(4)	3	0	10	13	NA	NA	NA	NA	NA
Birthrate/ cowyear (%)	6.4	0.0	4.9	4.5					
Mortality/ pod-year(%)	4.1	0.0	5.2	4.3					

1974-1981(3)		1973-1980
Size (First)	70	28
Size (Last)	79	34
Change	+10	+5
% change/year	+1.4	+2.5

1974-1977(3)		
Size (1974)	70	15
Size (1977)	77	13
Change	+7	-3
% change/year	+2.5	-3.3

(1) From Osborne et al. (1985)
(2) From Bigg (1982)
(3) Though data from southern pods are available from Osborne et al. (1985), all data are from Bigg (1982) in order to be consistent between regions.
(4) Results are tentative

APPENDIX B

Marine Mammal Necropsy Procedure

Necropsy forms were used to record all data.

1. Any external marks, wounds, pelage, sex, presence of umbilicus, and general condition were noted. We examined for scars, external parasites, lesions, and swelling. Any external abnormality was sampled for histopathology. Any flipper lesion was sampled for Leptospirosis.

2. The ventral and dorsal views of the animal were photographed. Anything unusual was photographed.

3. We measured length, weight, and girth. We measured umbilicus lengths if present on pups.

4. If pups were in good condition, we swabbed (dacron) the nose, throat, and anus for virology.

5. Oral cavity and nares were examined for blood, fluid, or blockage. Teeth were examined, development was noted for pups.

6. A mid-ventral incision was cut from anus to lower jaw. Blubber thickness was measured above the sternum. A blubber sample was taken for chlorinated hydrocarbon (Clhc) analysis.

7. The diaphram was examined for negative pressure in pleural cavity. The ribs were cut from sternum. Any presence of fluid or blood in the pleural cavity was noted.

8. For pups, the umbilicus artery and vein were examined for inflammation, pus, clotted blood or blockages. The umbilicus junction was sampled for histopathology.

9. Samples of the salivary glands, adjacent lymph node, thyroid, and thymus were collected for histopathology, any abnormality in size or structure were noted. The entire length of trachea and esophogus were examined for contents or blockages.

10. Blood was removed from heart with syringe and injected into a red top tube. The heart was examined; all chambers, aorta, and pulmonary artery for condition and parasites. A section was collected for histopathology.

11. The lungs were examined for growths or other abnormalities. If neonate, a portion of the lung was floated or examined for texture to determine aeration. A section was sampled for histopathology.

12. The pectoral lymphnode was sampled for histopathology, and measured if size appeared abnormal. Pectoral muscle was collected for ClHC analysis. Rib was sampled for histopathology by cutting a section and slicing longitudinally to expose marrow.

13. The liver was examined for lesions, abnormal size, or other abnormalities. The liver was collected for histopathology and swabbed (culturette) using sterile technique. For premature pups or animals with lesions, the liver was sampled for leptospirosis using sterile technique. Liver was sampled for heavy metal (HM) and for ClHC analysis.

14. The spleen was examined, noting any abnormalities. We sampled for histopathology, including a connected lymphnode, and sampled for HM.

15. For adults or subadult animals, the ends of the stomach were tied, removed, and frozen for food habits analysis. For pups, the stomach was opened and contents noted. The pancreas was collected for histopathology. The intestines and colon were examined for abnormalities. Meconium, fetal hair, or other stool or blockage were noted. The colon was sampled for histopathology.

16. The kidneys were examined. We sliced longitudinally, and examined for swelling, white tissue, cysts of other abnormalities. For premature pups, we collected tissue for Leptospirosis using sterile technique. Kidney was collected for histopathology and HM.

17. Adrenals were examined and sampled for histopathology. They were measured if they appeared abnormal in size.

18. The bladder was examined. For premature pups, the urine (if present) was collected with sterile syringe for Leptospirosis.

19. The reproductive tract was examined. For males, testes were sampled for histopathology. For female non-pups, both horns of the uterus were examined for embryo, fetus, scars, swelling, or occlusion. Both ovaries were examined for presence of corpra albicantia or luteum and fixed in 10% buffered formalin. The entire uterus was collected and frozen.

20. Tissue was removed from around the skull and the presence of contusions or fractures of the skull were noted. The skull was opened, and the brain swabbed (culturette) using sterile technique for bacteriology sampling. Brain tissue was collected for ClHC. Sections of the brain were sampled for histopathology. The pituitary gland was sampled for histopathology.

Special Sampling Techniques:

All samples were labelled with sample number, tissue type, and date collected.

ClHC: Chlorinated Hydrocarbons: All implements used were rinsed with methylene chloride prior to coming into contact with the sample, and between taking samples from different tissues. As large a portion as possible was sampled. This was to allow sub-samples to be taken from the unexposed interior of samples if required. The samples were collected into superclean glass jars with aluminum foil caps, and frozen at -15°C.

HM: Heavy metals were collected into sterile Whirl-Paks and frozen at -15°C.

Leptospirosis: Each sample was collected aseptically by searing the tissue surface with a hot spatula and removing a small sample of tissue from beneath the seared surface. If urine was present in the bladder, it was removed with a sterile syringe and 2 drops were deposited into the leptospirosis medium. Samples were shipped to Oregon State University.

Bacteriology: Bacterial samples were collected by swabbing with a culturette. For brain samples, an area on the surface was seared with a hot spatula and the culturette was punctured through this area. For liver, a section was seared with a hot spatula and opened with scalpel (rinsed with alcohol and then heated), then swabbed beneath the seared surface. If froth was present in the respiratory tract, it was also swabbed. Samples were stored at room temperature until they could be sent to Oregon State University.

Virology: The virology medium was refrigerated prior to sampling. Using dacron swabs, the mucous membrane of the nose, throat, and anus were swabbed and then placed into the virology medium. Samples were kept on ice or refrigerated until sent to Oregon State University.

Histopathology: All tissues were sampled into a large jar containing 10% buffered formalin in a 1:5-10 ratio of sample to formalin. A thin section was sampled to fix tissue in the formalin. All unusual lesions were cross-sectioned so that sections included both normal and abnormal tissues. Samples were stored until delivered to Evergreen Professional Services, Seattle, Washington.